NATIONAL CHILDREN'S STUDY RESEARCH PLAN

Volume 1

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THE NATIONAL CHILDREN'S STUDY

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VOLUME 1

TABLE OF CONTENTS

VOLUME 1

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

List of Tables

List of Figures

TABLE OF CONTENTS (continued)

VOLUME 2: APPENDICES

PRÉCIS

Background

Our nation has made great strides in reducing or eliminating classic childhood illnesses such as measles, mumps, and chicken pox. Significant advances have been made to improve child health and development by identifying the causes of many diseases; by developing preventive measures, treatments and cures; and by providing resources to support health care for children. Trends in childhood illnesses have emerged such as increased rates of childhood asthma, alarming numbers of children diagnosed with autism, and an obesity epidemic. This new childhood morbidity threatens to undo the progress made in child health promotion and disease prevention and to add a significant new cost burden to the economy.

In the late 1990s, numerous experts called for new data to better understand child health and development. In 1999, the President's Task Force on Environmental Health and Safety Risks to Children recommended a large longitudinal study of children to fill knowledge gaps about environmental influences on child health and development. The Children's Health Act of 2000 authorized and directed a consortium of federal agencies, led by the National Institute of Child Health and Human Development (NICHD) in partnership with the Centers for Disease Control and Prevention (CDC), the United States Environmental Protection Agency (EPA) and the National Institute of Environmental Health Sciences (NIEHS), to plan and to conduct the study.

The importance and timeliness of this study are based on factors that include the demonstrated and profound effects on child health of environmental exposures, such as lead in early childhood and alcohol during pregnancy; the special vulnerabilities of children to environmental exposures compared to adults; known ongoing exposures, such as prevalent levels of nonpersistent pesticides or hours of media exposure per day in young children; and evidence for environmental contributions to or causes of high-impact conditions, such as autism, developmental disabilities, asthma, and obesity. Science and technology have advanced to a point where it is possible to examine the individual and combined effects of genetic and environmental exposures, genetic variation, and multiple outcomes over life stages in the same individuals. The study design and data collected are determined by requirements necessary to test an integrated set of core hypotheses regarding the relations of environmental and genetic factors with priority outcomes in children, and later in adults.

Goal and aims

The goal of the National Children's Study (NCS) is to provide information that will ultimately lead to improvements in the health, development, and well-being of children. The primary aim of the NCS is to investigate the separate and combined effects of environmental exposures (chemical, biological, physical, and psychosocial) as well as gene-environment interactions on pregnancy outcomes, child health and development, and precursors of adult disease. In addition to this broad purpose, the Study has several specific goals:

(1) Determine the presence or absence of effects, both harmful and helpful, related to the timing, frequency, magnitude, and duration of specific chemical, physical, biological, and psychosocial exposures in children's environments from preconception to adulthood.

(2) Determine possible environmental contributions to, or causes of, specific diseases and conditions of children, including, but not limited to, prematurity and other outcomes

of pregnancy, neurological and developmental disorders, psychiatric and behavioral disorders, altered physical development and sexual maturation, obesity and insulin resistance, asthma, and injuries.

(3) Determine how genotypic variation and mechanisms, and the interaction of genes with environmental factors, influence disease risk and developmental trajectories in children.

(4) Serve as a national resource for future studies of child health and development by providing a rich database and repository of environmental and biological samples and information that can be used to address future questions and hypotheses.

Methods

This longitudinal cohort study will follow a representative sample of approximately 100,000 children born in the United States. Children will be followed from before birth until age 21.

Sampling and recruitment. The Study will employ a national probability sampling approach to select locations for conduct of the Study. The sampling design utilizes a multistage clustered approach. In the first stage, 105 locations (generally corresponding to single counties) were randomly selected from all U.S. counties. Seven of the locations will serve as the Vanguard Locations and will participate in the pilot phase of the Study. Because the focus of the Study includes assessment of the impact of exposures that occur early in pregnancy, both pregnant women and their partners and women of childbearing age comprise the initial target population for enrollment in the Study Locations. At the time of enrollment, participants will be asked to provide written informed consent for participation in the Study. Three distinct groups will be enrolled and followed: pregnant women and their partners; couples planning pregnancy; and women not currently planning pregnancy but with some probability of becoming pregnant during the four-year enrollment timeframe.

Follow-up. The initial follow-up of women enrolled in the study prior to pregnancy (the preconception cohort) will vary with each woman's probability of pregnancy. Women with a high probability of becoming pregnant (generally a subset of women trying to become pregnant) will receive an in-person visit and as many as three telephone contacts during the four months following enrollment. In contrast, women with the lowest probability of becoming pregnant will be contacted annually by phone. It is anticipated that during the enrollment period, a woman's probability of becoming pregnant will not be stagnant. Data collection schedules will be modified based on the most current information on each individual's probability of pregnancy.

Once women are pregnant, follow-up visit schedules will be identical for all women and children enrolled in the Study regardless of the initial probability of pregnancy. A minimum of six in-person visits are planned from the first trimester of pregnancy through age 3. Three of these visits are in the home, and three are in a clinical setting (one of which is the place of delivery of the infant). After age 3, in-person visits should occur every two to three years with an additional home visit after each change of permanent residence. Remote data collections (e.g., telephone, computer, or mail-in questionnaires) will occur between face-to-face contacts. The expected frequency of contact (face-to-face or remote) is approximately every three months through age 1, every six months through age 5, and annually thereafter. For a sample of children enrolled in the Study, visits will also be made to child care and school settings for collection of environmental samples and observational data.

The schedule of in-person contacts and phone interviews from pregnancy through age 21 are outlined in the table below:

Timing and Location of Study Contacts

Age of child	Location of visit
Preconception	As outlined above
1st trimester	Home
2nd trimester	Phone
3rd trimester	Clinic
Birth	Place of delivery
3 months	Phone
6 months	Home
9 months	Phone
12 months	Home
18 months	Phone
24 months	Phone
30 months	Phone
3 years	Clinic
5 years*	To be decided
7 years	To be decided
9 years	To be decided
12 years	To be decided
16 years	To be decided
21 years	To be decided

*Timing and location of visits from 5 years onward is provisional

Anticipated biologic specimens include blood, urine, hair, and nail clippings from mothers and children; blood, urine, and hair from fathers; cord blood, umbilical cord and placental tissues, and meconium collected at or around the time of delivery; vaginal swabs, and breast milk from mothers. Anticipated environmental samples include air, dust, soil, food, and water.

Expected contributions of the Study

The NCS is in a unique position to answer questions regarding the effects of environmental exposures on the long-term health of children. The focus on exposures prior to and early in pregnancy and the breadth of planned exposure and outcome measurements are unique features of the Study. As technology evolves, stored data specimens (biologic and environmental) will provide a valuable resource to answer questions for future generations.

The Study's prospective longitudinal design will permit an in-depth examination of the effects of environmental exposures as they unfold over the course of development. This will include an unprecedented, process-oriented understanding of how exposures at particular points in development lead to both immediate and long-term consequences for children, and what circumstances, characteristics, or genetic predispositions mediate or moderate the relation between exposure and outcome. The size and representative nature of the sample will permit both valid inferences about the U.S. population as a whole and exploration of subgroup-specific patterns of adaptation and maladaptation.

The data collected for the NCS will also provide a platform for future research. Data and biological and environmental samples will be available for future studies as science evolves and new questions arise. The NCS will serve as an exceptional resource both for science and for society.

PART I
BACKGROUND AND SIGNIFICANCE

Chapter 1

Background

PART I: BACKGROUND AND SIGNIFICANCE

1. BACKGROUND

Patterns of illness among children in the United States and other industrially developed nations have changed substantially during the past 100 years (Bloom & Dey, 2004). Before and during the first half of the last century, infectious disease comprised the principal threat to children. In contrast, the major illnesses and disorders that impair health, growth, and development today are chronic conditions stemming from the complex interaction of environmental exposures and inherent genetic factors. Some label this the "new pediatric morbidity" (Haggerty, 1975). These conditions include: premature births (Ananth, Joseph, Demisse, & Vintzileos, 2005); asthma (Mannino et al., 2002); injuries (Thornton, Craft, Dahlberg, Lynch, & Baer, 2002); childhood cancer (Linet, Ries, Smith, Tarone, & Devesa, 1999); neurodevelopmental disorders, such as learning disabilities, dyslexia, mental retardation, attention deficit/hyperactivity disorder, and autism (Boyle, Decoufle, & Yeargin-Allsopp, 1994; Newschaffer, Falb, & Gurney, 2005; Scahill & Schwab-Stone, 2000; Shaywitz, 1998); obesity and type 2 diabetes (SEARCH Writing Group, 2004); birth defects such as hypospadias (Paulozzi, 1999); and cardiac defects (Towbin et al., 2006). Addressing the causes and contributors to these and similar chronic conditions is the major challenge to public health practitioners and pediatric researchers today, and constitutes the frontier that must be crossed if the health and well-being of children in developed countries is to move forward. The National Children's Study is designed to address these issues with robust science and the ability to generalize the data to the U.S. population.

The National Children's Study's design rests on the principle that both health and susceptibility to disease are determined by dynamic processes that occur throughout life. Perturbations ("insults") that impact health states may occur any time from preconception through adult life. These insults can affect viability, differentiation of major organ systems, somatic growth, and the development of functional processes including maturation of metabolic systems. A range of determinants acting either in concert or synergistically may impact growth and development. These include the built and natural environments with their chemical and physical factors, the social environment, individual behaviors, and biological factors including genetics. Of particular importance are the earliest stages of human development, pregnancy and early childhood, when cell division, differentiation, and maturation are most rapid.

These health determinants may influence development in many ways. For those with high potency when acting at critical periods of development, such as thalidomide or Accutane, severe birth defects will result in most exposed offspring. Most environmental factors, however, are not so potent. More often, factors operating at critical or sensitive periods of development will interact with other factors over the life course to raise or lower the risk of adverse health outcomes. These factors may be genetic or non-genetic. For example, accelerated weight gain during childhood is associated with increased risks of diabetes and cardiovascular outcomes later in life; this phenomenon is accentuated among children born with restricted fetal growth (Barker, 2005; Bhargava et al., 2004). The risk of orofacial clefts due to maternal cigarette smoking is markedly increased in children with certain genetic traits and/or reduced folic acid intake (Lammer, Shaw, Iovannisci, & Finnell, 2005; Shaw et al., 2005; Shaw, Wasserman, Murray, & Lammer, 1998). Only with this appreciation of the complexity of interactions among genetic and environmental factors will we be able to inform the next generation of caregivers about effective prevention and treatment to lower the burden of common chronic conditions of childhood and later-onset diseases that arise from early developmental insults.

1.1 The Children's Health Act of 2000

Faced with the challenge of how to address the potential risks of many environmental factors that may be affecting the health and development of children, the President's Task Force on Health Risks and Safety Risks to Children concluded in 1999 that a large study to define the actual risks associated with broad environmental exposures is an essential first step. Following the recommendation of the task force, the U.S. Congress passed the Children's Health Act of 2000, which directed the National Institute of Child Health and Human Development (NICHD) to conduct a national longitudinal study of environmental influences on children's health and development. The National Institute of Environmental Health Sciences (NIEHS), the Centers for Disease Control and Prevention (CDC), and the U.S. Environmental Protection Agency (EPA) joined the NICHD in planning this study.

The Children's Health Act of 2000 (Public Law 106-310 Sec. 1004) specified that the study should extend from the prenatal period to adulthood, following a sample of children across development. It should investigate the short-term and long-term influences of physical, chemical, biological, and psychosocial environmental exposures on children's health and development, including not only physical health, but behavioral, emotional, and educational outcomes as well. The study should elucidate both those factors that protect children from detrimental outcomes and those that put them at risk, including sufficient diversity to address the existence and consequences of health disparities among children in the United States. The scientific rationale for this program of research, now named the National Children's Study, is described below.

1.2 Rationale for the National Children's Study

1.2.1 The Public Health Burden of Childhood Chronic Conditions

While there are many important conditions of childhood that have grave effects on certain individuals and families, there are some that also place a great burden on the population because of their prevalence, severity, and/or cost. For example, there are increasing concerns about the large (and perhaps growing) number of American children who have one or more major chronic health or developmental conditions. As many as 17 percent of children have some type of developmental disorder (Boyle et al., 1994), about 21 percent have a diagnosable mental or addictive disorder with at least minimum impairment (U.S. Department of Health and Human Services, 2000), and about 7 percent have asthma (Mannino et al., 1998). The NCS is particularly poised to examine these conditions because it is a large study of the general population. Through the extensive planning process of the NCS, the following areas emerged as primary outcomes around which the Study's core hypotheses have been generated: pregnancy outcomes; neurodevelopment and behavior; asthma; obesity and growth; injury; and reproductive development. Additionally, the NCS is committed to assessing predictors of healthy development. The data collection process will also allow the examination of a range of health outcomes that extend beyond those identified in this Study.

The priority outcome areas were chosen not only because of their importance to public health, but also because a research study of the scope and magnitude of the NCS is required to understand their origins and course. Since many of the outcomes may arise as a consequence of in utero exposures, study of these outcomes must begin before birth. Additionally, a variety of exposures likely contribute directly, indirectly, and interactively to these outcomes. A full understanding of their etiology requires a study covering a range of exposures. Genetics could also play a role both in the origin and expression of disorders, thus a complete study must include an exploration of direct genetic contributions and of gene-environment interactions. Furthermore, each outcome has a meaningful range of manifestations over the course of development, including sensitive periods for exposures, different ages of onset, and changes in

nature or severity over development. Only a longitudinal study can track these outcomes as they unfold during childhood and adolescence. Finally, to examine these exposure-outcome relations in a definitive manner, the Study must have sufficient power, and thus sufficient sample size, to explore both normative and low-prevalence outcomes. The NCS will follow a representative sample of 100,000 children from before birth to age 21 and will include assessments, collected through a variety of modalities, of chemical, physical, psychosocial, and biological exposures, as well as genetics. Incorporating both breadth and depth of investigation, the NCS will be particularly well suited to provide scientists and practitioners with the tools to address these new childhood morbidities, and to promote health and well-being in our children.

1.2.1.1 Pregnancy Outcomes

Low birth weight and preterm delivery are highly correlated and continue to be among the major refractory causes of infant mortality and childhood morbidity (Gutbrod, Wolke, Soehne, Ohrt, & Rigel, 2000). Identified environmental factors for increased risk of preterm birth, which include maternal smoking (Kyrklund-Blomberg, Granath, & Cnattingius, 2005), chemical agents (Gonzales-Cossio et al., 1997; Hinckley, Bachand, & Reif, 2005), infection (Andrews, Hauth, & Goldenberg, 2000; Pararas, Skevaki, & Kafetzis, 2006; Romero, Espinoza, Chaiworapongsa, & Kalache, 2002), stress (Hobel, 2004; Park, Park, Lockwood, & Norwitz, 2005), and even air pollution (Rogers & Dunlop, 2006), all point to environmental exposure etiologies. More recent reports point to more complex interactions between environmental and genetic factors. Several possible genetic variations have been described that place some women at particular risk of premature births with certain exposures, such as infection (Varner & Esplin, 2005) and cigarette smoke (Wang et al, 2002).

Birth defects are the leading cause of infant mortality and are responsible for more than 8,000 (approximately 20 percent) of the 40,000 infant deaths that occur annually (CDC, 1998). Following on the morphologic birth defects of fetal exposure to alcohol (Jones, Smith, Ulleland, & Streissguth, 1973), there are concerns about other birth defects such as hypospadias that have increased in recent years along with exposures to phthalates and other endocrine active compounds (Paulozzi, 1999; Rogan, Gladen, Guo, & Hsu, 1999; Weiss, 2002). There are also concerns about central nervous system defects, such as anencephaly, spina bifida, and hydrocephaly, and their association with diabetes, with lesser alterations possibly associated with altered glucose metabolism (Anderson et al., 2005).

1.2.1.2 Neurodevelopment and Behavior

In contrast to birth defects which are structural in nature, developmental disabilities are recognized because of abnormalities in functioning that emerge as a child ages. Almost 20 percent of all children in the United States are reported to have some type of developmental disability (Boyle et al., 1994), including approximately 2 percent of school-age children with a serious developmental disability (Crain, 2000). Conditions that are representative of developmental disabilities include mental retardation, cerebral palsy, attention deficit/hyperactivity disorder (ADHD) and autism. Numerous exposures in utero and during infancy, most notably lead (Lidsky & Schneider, 2003), alcohol (Mattson & Riley, 1998), and nurturing (Bradley et al., 1989), have been identified as affecting neurological and cognitive development. The causes of most cases of mental retardation, however, are unknown (Yeargin-Allsopp, Murphy, Cordero, Decoufle, & Hollowell, 1997). Recent evidence reveals the potential contribution of known neurodevelopmental toxicants to developmental disabilities at levels of exposure well below currently recognized levels of toxicity (Lanphear, Vorhees, & Bellinger, 2005; Schober et al., 2003). Previously unidentified environmental agents, including persistent and nonpersistent pesticides as neurotoxicants, may also play a role in developmental disabilities (Kofman, Berger, Massarawa,

Friedman, & Jaffar, 2006; Rice & Barone, 2000; Weiss, 2000). The cost of diminished child functioning due to environmental toxicants is substantial (Grosse, Matte, Schwartz, & Jackson, 2002; Salkever, 1995; Weiss, 2000).

The etiology of neurodevelopmental disorders can be complex and difficult to specify. For example autism is a neurodevelopmental disorder that was once believed to be rare (i.e., 4-5 per 10,000 children); however, the number of individuals receiving services for autism has increased dramatically in the past 10 years. The current prevalence of autism and the broader group of autism spectrum disorders stands at about 3-6 per 1,000 children (Gillberg & Wing 1999; Hirtz, 2000; Rutter, 2005; Yeargin-Allsop et al., 2003). Although autism has a strong genetic component (The Challenges of Autism, 2000), environmental and social factors are also thought to play a significant role in its expression, and a number of environmental agents have been suspected of interacting with genetic factors to cause the apparent increase of autism.

1.2.1.3 Child Health and Development

In addition to investigation of specific disorders of childhood, an understanding of child health and development involves examination of individual differences and children's trajectories through time on measures of health, well-being, social and emotional development, and cognitive development and achievement.

Early developmental deficits can compromise subsequent social and academic success. While most children enter kindergarten having mastered basic skills, a significant percentage lags behind in key domains. Between 18 to 42 percent of preschoolers are estimated to lag behind their classmates significantly in their preparedness for learning (West, Denton, & Germino-Hausken, 2000). In the realm of behavior and conduct, approximately 12 percent of infants and toddlers have significant behavioral or emotional problems (Briggs-Gowan, Carter, Skuban, & Horwitz, 2001). Such problems unfold in complex ways over time, however, as research indicates that less than 50 percent of children with conduct problems during the toddler or preschool period continue to have significant problems one to two years later (Baillargeon et al., 2007; Lavigne et al., 1998). Additionally, children who show early signs of social competence tend to become even more prosocial with development (Baillargeon et al., 2007). Nonetheless, many children with deficits in emotional, social, and cognitive skills at school entry are likely to have both ongoing conduct problems, and difficulties with academic achievement (Wentzel & Asher, 1995).

Many different experiences and exposures have the potential both to affect child health and development at sensitive periods and to change children's developmental trajectories. For example, sensitive parenting and secure infant-parent attachment during infancy predict children's subsequent competence and healthy social functioning (Thompson, 1999). In contrast, parental mental health problems can lead to disturbances in parent-child interactions (Jameson, Gelfand, & Kulcsar, 1997), and the strategies that a young child uses to relate to a mentally distressed parent can become persistent, resistant to change, and can develop into a long-term behavioral pattern of response (Field, 1995; Lyons-Ruth, Wolfe, Lyubchik, & Steingard, 2002). Contexts outside the family can also have great impact on children. Early experience in high-quality center-based child care predicts better vocabulary skills, but also slightly elevated aggressive behavior in middle childhood (Belsky et al., 2007). The sensitive periods for exposure and trajectories of functioning are multifaceted and are also likely moderated by genetic and physiological factors (Curtis & Cicchetti, 2003). Longitudinal research with a sufficiently large and representative sample is needed to untangle these intricate pathways.

1.2.1.4 Asthma

Among children, asthma is the most common chronic illness (National Academy of Sciences, Institute of Medicine, 2000). Asthma prevalence in the United States, estimated from the National Health Interview Survey (NHIS) by the American Lung Association (American Lung Association Epidemiology and Statistics Unit, 2006), shows the prevalence of asthma increased 85 percent from 1982 through 1996 to an estimated 14.6 million persons (55.2 per 1,000). This increase was 76 percent in children younger than 18, or 4.43 million persons in 1996 (62.0 per 1,000). This trend paralleled increasing asthma hospitalization and death rates in children (Akinbami, 2006; American Lung Association Epidemiology and Statistics Unit, 2006). In 2004, the prevalence of doctor-diagnosed asthma reached 30.2 million Americans (104.7 per 1,000), including 6.5 million children younger than 18. Almost 4 million children younger than 18 were estimated to have experienced an asthma attack in 2004. Prevalence data in the United States, both from the 12-month prevalence (before 1997) and 12-month attack prevalence of asthma (since 1997), were highest among children ages 5-14, higher among Blacks compared with whites, and higher among females than males (Akinbami, 2006; American Lung Association Epidemiology and Statistics Unit, 2006). Approximately 38 percent of the hospital discharges related to asthma in 2004 were in children younger than 15, although only 21 percent of the U.S. population was younger than 15.

Asthma is associated with substantial physical and behavioral disability among children. Thirty percent of children with asthma reported activity limitation compared to 5 percent of children without asthma, and asthma was estimated to account for 10 million missed school days and 13 million physician contacts among children in 1988 (Taylor & Newacheck, 1992). This is an underestimate of the current burden because of increasing trends in asthma prevalence and associated morbidity (Mannino et al., 2002). The annual estimated cost of pediatric asthma in the United States in 1997 was $6.6 billion (Landrigan, Schechter, Lipton, Fahs, & Schwartz, 2002).

In 2004, the total cost of asthma was estimated at $16.1 billion, including $11.5 billion in direct health care costs and $4.6 billion in indirect costs (lost productivity) (National Institutes of Health, 2004). The severe forms of asthma account for a disproportionate amount of the direct costs. Malone, Lawson and Smith (2000) estimated that less than 20 percent of asthmatics account for more than 80 percent of the direct costs. Asthma also poses a substantial and increasing public health burden because of school absences and restriction of children's usual physical and social activities (Newacheck & Halfon, 2000).

Asthma is a complex disease characterized by pulmonary obstruction due to inflammatory response within central and peripheral airways. Asthma has a variety of clinical phenotypes, which carry implications for disease etiology, evolution, and severity (Martinez, 2000; Martinez & Helms, 1998). Current understanding of the etiology and severity of asthma focuses on individual response to a range of interacting immunogenic and immuno-protective factors (Busse & Lemanske, 2001): air pollution and bioaerosols (including allergens, endotoxin, and mold); respiratory tract infections; maternal stress; dietary antioxidants; and early exposure to bacterial and microbial products. This focus opens a range of potential research areas that address interactions between host response (e.g., individual inflammatory response, genetic makeup), potential inflammatory triggers (e.g., ozone, particulate matter, and other airborne pollutants; viral infection; animal or fungal antigens), and potential protective factors (e.g., early exposure to bacterial endotoxin, dietary antioxidants).

1.2.1.5 Obesity and Growth

The prevalence of overweight among children is greater than 15 percent among children age 6 or older, and this prevalence has increased during the past 40 years (Ogden, Flegal, Carroll, & Johnson, 2002). Being overweight as a child is a risk factor for being overweight as an adult (Serdula et al., 1993) and is associated with increased risk of type 2 diabetes, hypertension, and coronary artery disease (Freedman et al., 2001). Being overweight as a child also increases the risk of developing type 2 diabetes before age 21 (Sinha et al., 2002).

The best estimate of the prevalence of type 2 diabetes among those younger than 21 in the United States is about 0.1 percent based on National Health and Nutrition Examination Survey (NHANES) data from 1988-1994 (Fagot-Campagna, Saaddine, Flegal, & Beckles, 2001). Given the increase in overweight among children, it seems reasonable to assume that the prevalence now is higher than 0.1 percent—but by how much is unclear. Although type 2 diabetes may not be common enough for the NCS to examine with sufficient power, insulin resistance or closely related conditions, such as metabolic syndrome, are outcomes that would occur with sufficient frequency among subjects younger than 21 and could serve as both outcomes and markers for adult disease. Insulin resistance is considered the underlying abnormality in metabolic syndrome. Metabolic syndrome, according to the World Health Organization and as modified by Laaksonen et al. (2002), is defined by fasting hyperinsulinemia, impaired fasting glycemia or diabetes, and the presence of at least two of the following: abdominal obesity, dislipidemia (hypertriglyceridemia or low HDL cholesterol), or hypertension. Such a definition is feasible for detection in large-scale epidemiologic studies and identifies those who are at high risk of developing type 2 diabetes. The prevalence of metabolic syndrome among adults, as compared with the prevalence of type 2 diabetes, is about four-fold greater (Laaksonen et al., 2002). Investigations based on NHANES III data indicated that approximately 4 to 10 percent of adolescents ages 12-19 have metabolic syndrome (Cook, Weitzman, Auinger, Nguyen & Dietz, 2003). Thus, it is reasonable to assume that an outcome definition of metabolic syndrome like the definition presented above would have a prevalence rate above 0.2 percent. This means the NCS would have sufficient power to examine metabolic syndrome in relation to a wide range of exposure levels.

Mounting evidence suggests prenatal factors and early childhood experiences may influence the development of disease later in life (Barker, 1992). Altered fetal growth has been related to increased risk of cardiovascular disease, hypertension, and diabetes in adulthood (Barker, 1995; Barker & Osmond, 1986; Barker, Winter, Osmond, Margetts, & Simmonds, 1989; Poulter, Chang, MacGregor, Snieder, & Spector, 1999). Accelerated childhood growth is related to the risk of breast cancer in women (Ahlgren, Melbye, Wohlfahrt, & Sorensen, 2004) and to impaired glucose tolerance in adulthood (Hales et al., 1991).

1.2.1.6 Injury

Both unintentional injuries (e.g., motor vehicle crashes, suffocations) and intentional ones (interpersonal violence, child maltreatment, self-inflicted injuries) exert a tremendous toll in childhood. Beyond the first year of life, unintentional injuries are the leading cause of mortality in every age group until age 44 years (Centers for Disease Control and Prevention, National Center for Injury Prevention and Control, 2007). In the teen years, homicide and suicide are the second and third leading causes of death, respectively. Fatal injuries represent only a small portion of the problem; it is estimated that in 2001 more than 230,000 children younger than 21 were hospitalized for an injury and approximately 9.7 million were treated in an emergency room and released (Centers for Disease Control and Prevention, National Center for Injury Prevention and Control, 2007). The economic burden of injuries for persons of all ages was estimated at $406 billion in 2000, including $80.2 billion in medical care costs and $326 billion in

lost productivity (Finkelstein, Corso, & Miller, 2006). For children and adolescents younger than 14, the total economic burden in 2000 was estimated at more than $50 billion.

Similar to other outcomes, injuries result from exposures in multiple domains and represent the convergence of individual behaviors (e.g., risk taking, aggression), the physical environment (e.g., road embankment, access to weapons), and societal factors (e.g., access to emergency care). Many serious injuries result in significant impairment with lifelong consequences for health and development. From this perspective, injuries are not only an important outcome to investigate in the NCS, but also an exposure that alters trajectories of development in multiple outcome domains of interest. This could occur through direct effects (e.g. a head injury causing direct brain damage) or through more subtle pathways (e.g., the emotional effects of the event leading to post-traumatic distress; changes in level of physical activity due to physical limitations imposed by injury).

Haddon and other injury prevention pioneers conceptualized injuries as the consequence of human exposure to energy in ways that resulted in an injury (Haddon, 1964; Stapp, 1957). This idea expanded the field to analysis and study of physical forces and how to modify their impact on humans as a conceptual framework for the control and prevention of injuries (Haddon, 1970). Identification of the combination of individual, environmental, and societal factors that result in injury is critical for the development of effective interventions. Childhood injury prevention experts recommended conducting longitudinal cohort studies to identify environmental risk and contextual factors and understand how they can be modified to reduce injuries (Committee on Injury and Poison Prevention, 1996; Scheidt, 1988). Careful analyses of multiple conceptual frameworks for injury prevention emphasize that a temporal perspective and acknowledgement of the complex interplay of societal and environmental factors are critical (Andersson & Menckel, 1995). Thus, moving beyond the surveillance and cross-sectional methodologies (Scheidt et al., 1995) to longitudinal studies of sufficient size is essential to separate confounders and isolate causal relations that can provide the basis of effective preventive strategies (Rivara, 1999).

1.2.1.7 Reproductive Development

Hypospadias is one of the most common congenital anomalies, affecting 27-55 of every 10,000 births in the United States (Paulozzi, 1999; Paulozzi et al., 1997) or 0.8 percent of male live births (Pohl et al., 2007). Cryptorchidism affects 3 percent of full-term male newborns (up to 7.7 percent of low birth weight infants) decreasing to about 1 percent by age 1 (Pohl et al., 2007). Reports of increasing trends for hypospadias (Paulozzi, 1999; Paulozzi et al., 1997) and cryptorchidism (Paulozzi, 1999) in the United States and other countries and secular trends toward decreasing age at menarche and other measures of puberty onset in boys and girls (Herman-Giddens et al, 1997; Herman-Giddens, 2006; Kaplowitz et al., 2001; Lee et al., 2001), have created concerns about the etiological factors behind these trends. These factors include better nutrition or perhaps over-nutrition; earlier and greater growth; increasing incidence of obesity; and socioeconomic or environmental factors.

Documented exposures of children and pregnant women to compounds that have potential reproductive toxicity support the importance of studying environmental determinants of age at puberty. Exposure of children and pregnant women to hormonally-active agents (HAAs, also called endocrine disruptors) is widespread in America (CDC, 2003), and animal studies suggest the potential for toxicity at current levels of exposure (Vom Saal & Hughes, 2005). For example, cross-sectional data from NHANES III (Selevan et al., 2003; Wu et al., 2003) suggest that higher blood lead levels may be associated with a delay in the onset of puberty in girls, paralleling similar findings in animals. Precocious puberty was reported in girls who were both exposed in utero to the fire retardant FireMaster, which contained polybrominated biphenyls (PBBs), and breast-fed by mothers who were exposed to the fire retardant

(Blanck et al., 2000). Bisphenol A, a weak estrogen (Pottenger et al., 2000), is a high production volume chemical used in a variety of applications, including manufacturing flame retardants, resins, and plastics. Human exposure may arise in a number of circumstances, for instance, when foods are contaminated by heated plastics. Blood levels of bisphenol A in pregnant women (Schonfelder et al., 2002) are similar to those found in pregnant rats that give birth to offspring with bisphenol A-induced reproductive toxicity (Howdeschell, Hotchkiss, Thayer, Vandenbergh, & vom Saal, 1999; Pottenger et al., 2000; Rubin et al., 2001). Atrazine is a widely used herbicide. In a population-based probability sample of children ages 3-13, about 3 percent of children had detectable levels of an atrazine metabolite in their urine, and urban-rural differences in levels were not statistically significant. Recent experiments in peripubertal rats show that atrazine in doses of 30 milligram per kilogram orally per day for as long as 25 days delayed the onset of puberty (Ashby et al., 2002). It is not clear if the doses effective in animal experiments result in urinary metabolite levels like those seen among children with detectable levels.

Lack of accurate information on the level and timing of past exposures to HAAs has limited most previous studies of the potential human impacts of known and suspected HAAs. This limitation will be directly addressed by the prospective design of the NCS because exposures to chemicals will be measured during pregnancy, in breast milk, and in the perinatal period before the appearance of health effects. The measurement of multiple outcomes related to single, multiple, and continuous or repeated exposures is only possible with a large longitudinal study. The potential for cumulative effects on the reproductive system can only be discerned through the use of a large longitudinal sample that allows repeated measures of exposure and evaluation of reproductive outcomes through time. Measures or biomarkers of exposure are available for most HAAs of interest and will allow linking of exposures at specific life stages with early or late reproductive outcomes. Measures of gene prevalence and gene expression will permit examination of genetic polymorphisms that may influence gene-environment interactions and will allow assessments of genetically determined inter-individual differences in susceptibility to HAAs.

Since the effects of HAAs are gender specific, it will be necessary to study exposure-outcome links separately in males and females, which will reduce the sample size for each case to approximately 50,000. Susceptible subgroups related to genetic polymorphisms may require additional subgroup studies.

1.2.2 Environmental Factors That May Influence Childhood Chronic Conditions

This section provides the rationale for emphasis of the NCS on an array of environmental factors and their impacts across domains and time. These include the natural and built environments with their attendant chemical, physical, and biological factors; the social environment; individual behaviors; biological factors; and genetics. It should be emphasized that health states are determined by interactions among genetic and non-genetic factors, and that these interactions may change over time.

1.2.2.1 Chemical Exposures

There is increasing and ample evidence that children experience a significantly greater vulnerability to the effects of chemical exposures than do adults in similar environments (Anderson, Diwan, Fear, & Roman, 2000; International Programme on Chemical Safety [IPCS], in press). A National Academy of Sciences Committee on Pesticides in the Diets of Infants and Children identified four fundamental differences that contribute to children's heightened susceptibility to toxic chemicals (National Research Council, 1993): (1) Children have disproportionately heavy exposures to environmental toxicants as a consequence of their greater intake kilogram-for-kilogram of food, water,

and air coupled with their unique behaviors, in particular their oral exploratory behavior in infancy; (2) Children's metabolic pathways, especially in the first months after birth, are immature. In many instances, children are less able than adults to excrete and/or detoxify toxic compounds; (3) Children are undergoing rapid growth and development, which makes them more vulnerable to environmental toxicants; (4) Children have more years ahead of them to develop chronic diseases that may be initiated by their exposures than do adults. Although broad windows of sensitivity during development can be identified for many systems, information on exact timing of sensitivity, and on any preventable factors, is limited. This lack of information reinforces the importance of detailed exposure assessment.

The chemical environment in which children live has also changed with regard to known risks of several decades ago (Lioy, 1999; National Research Council, 1991). Today there are more than 80,000 synthetic chemicals, most developed since the 1950s (Environmental Protection Agency [EPA], 1998a). These include plastics, pesticides, fuels, building materials, antibiotics, chemotherapeutic agents, flame retardants, and synthetic hormones. Children are at especially high risk of exposure to the 2,800 synthetic chemicals produced in quantities of one million tons or more per year (Environmental Protection Agency, 1998b). These high-production-volume (HPV) chemicals are the synthetic materials dispersed most widely in air, food, water, and consumer products in homes, schools, and communities (EPA, 2001). Recent national surveys show quantifiable levels of HPV chemicals have been detected in the bodies of most Americans as well as in the milk of nursing mothers (EPA, 2003).

Although much remains to be learned about associations between the environment and disease in children, accumulating evidence suggests chemical, physical, and biological factors contribute to disease causation and severity. Numerous pollutants in the indoor environment—second-hand tobacco smoke, mold and mites, cockroach droppings, animal dander, and certain pesticides (CDC, 2005; Gergen et al., 1999)—have been identified as triggers for childhood asthma. Reduction in children's exposures to these indoor pollutants has been shown to reduce frequency of asthma (Lioy, Freeman, & Millette, 2002). Evidence indicates that ambient air pollutants—fine particulates, ozone, oxides of nitrogen, and diesel exhaust—also increase the incidence of asthma and trigger asthmatic attacks (Kattan et al., 2005; Salam, Li, Langholz, & Gilliland, 2004). Reduction in ambient air pollution has been associated with reduction in the number of hospitalizations due to asthma and other respiratory diseases (Friedman, Powell, Hutwagner, Graham, & Teague, 2001; Gauderman et al., 2004; Suh, Bahadori, Ballarino, & Spengler, 2000). Drinking water may have low-level concentrations of a number of chemical contaminants, such as pesticides, phthalate plasticizers, and byproducts of water disinfection. Animal studies indicate that some of the phthalate plasticizers have anti-androgenic properties and may cause birth defects (Blount et al., 2000; Barlow et al., 2003). Childhood cancer has long been linked to ionizing radiation. More recently, benzene, 1, 3-butadiene, and pesticides have been etiologically associated with childhood malignancies (Andrade et al., 2006; Daniels et al. 2001). A recent National Academy of Sciences study suggests that at least 28 percent of developmental disabilities in children may be caused by environmental contaminants acting alone or in combination with genetic factors (Bigbee et al., 1999; Lee, Cantor, Berzofsky, Zahm, & Blair, 2004; National Academy of Sciences, Committee on Developmental Toxicology, 2000; Slotkin, 1999). Although the concentrations of such contaminants may not be sufficiently high to cause overt acute toxicity among exposed individuals, the safety of low-level exposures to such chemicals in utero or during early childhood is unclear and is a serious concern.

The various routes and patterns of exposure in the environment can impact internal absorption and biological effects (EPA, 1998). Very little research is available about differences in patterns of exposure (e.g., short-term, peak, cumulative, chronic, or intermittent). The importance of considering exposures to a single or mixture of chemicals through all relevant pathways and routes as an aggregate exposure is exemplified by studies of chorpyrifos (IPCS, in press), lead (Albalak et al., 2003; Garcia Vargas et al., 2001; Morgan et al., 2005), arsenic (Pineda-Zavaleta et al. 2004), and DDT for malaria control (Carrizales et al., 2006; Diaz-Sanchez, Rumold, & Gong, 2006). These studies report high

levels of exposure from multiple routes with the largest contributor sometimes resulting from unexpected sources. How different patterns of exposure, such as peak exposure or cumulative exposure from all sources and pathways through time, determine the overall risk to individuals is not well understood (Herrera, Ochoa, Franco, Yanex, & Diaz-Barriga, 2006). Some studies of organophosphate pesticides (OP) point to greater susceptibility to cumulative exposure to OPs in children compared to adults (IPCS, in press). Thus far, however, the measurement methodology, capability, and adequate study size have not been available in any study to begin to understand the impact of routes and patterns of exposures (Wessels, Barr, & Mendola, 2003).

1.2.2.2 Physical Exposure: The Built Environment

The physical environment in which children live has prompted concerns about potential health effects. A higher proportion of children in America live in cities and suburbs than ever before, and the built environment has been shown to be capable of influencing children's physical and mental health and their risk of disease (EPA, 2003; Department of Agriculture, 2003; Frumkin, 2002; Galvez, Frieden, & Landrigan, 2003; Horowitz, Colson, Hebert, & Lancaster, 2004; Jackson, 2003). The adverse effects of the modern built environment are magnified in low-income, predominantly minority, urban communities where crowded streets, lack of outdoor play-spaces, limited access to fresh and healthy food, and substandard housing contribute to substantial and well-documented disparities in health (Morland, Wing, & Diez-Roux, 2002; Morland, Wing, Diez-Roux, & Poole, 2002; Sallis, Bauman, & Pratt, 1998; Sallis, Kraft, & Linton, 2002; Sallis et al., 1990). Recognition is increasing that characteristics of the built environment may influence diet and activity patterns and, as a result, increase the risk of obesity (Ewing, Schmid, Killingsworth, Zlot, & Raudenbusch, 2003; Frank, Andressen, & Schmid, 2004). Humpel et al. (2002) observed that physical environmental factors show consistent associations between the built environment and physical activity behavior. They also noted that availability of and access to bicycle paths, footpaths, health clubs, and swimming pools, as well as favorable aesthetics (e.g., indicating that it is pleasant near the home) are associated positively with physical activity. Thus, the physical environment is an important predictor of physical activity change and related health outcomes (Berrigan & Troiano, 2002; Berrigan, Troiano, McNeel, Disogra, & Ballard-Barbash, 2006). Physical activity of youth appears to be determined by many factors, including the physical environment, but the long-term influence of the built environment on children's physical activity is largely unexplored with about 75 percent of the extant literature being cross-sectional in nature (Sallis, Prochaska, & Taylor, 2000). A more recent review shows that physical activity in childhood exerts its strongest influence in diseases that have in common altered stress, inflammation, and leukocyte function, such as asthma and arthritis (Schwarzenberg & Sinaiko, 2006; Van Gaal, Mertens, & De Block, 2006). The impact of physical activity on critical periods of development in children need not be limited to the walking child, since assisted exercise in preterm infants has been shown to increase body weight and improve bone strength.

1.2.2.3 The Psychosocial Environment

The psychosocial environment plays a critical role in healthy development. Substantial evidence points to the complex and dynamic role that psychological and social environmental influences play in development, and in the creation and amelioration of health disparities. Concentrated poverty, racial segregation, and high levels of crime contribute to poor health, developmental deficits, and high levels of risk behaviors among individual residents (Aneshensel & Sucoff, 1996). Yet to be explored are the mechanisms and the interactions with genetic and other exposure factors needed to guide interventions. National and local public policies influence the resources available to individuals and families and their ability to manage health-related aspects of their lives. The functioning of families, the

most crucial element of the psychosocial environment for young children, is affected by economic, policy, social, and cultural dimensions of the environment.

Evidence and practical experience attest that parenting practices, as just one critically important component of a child's psychosocial environment, can have a profound impact on a child's development and outcome (Borkowski, Ramey, & Bristol-Power, 2002). There is an increasing body of evidence based on animal research which elucidates pathways that explain how early social environment can cause lasting changes in gene expression which remain into adulthood (Barr et al., 2004; Newman et al., 2005). It is also known that abuse and unstable parent-child relationships can lead to behavioral disorders and increased morbidity and mortality (Shonk & Cicchetti, 2001; U.S. Department of Health and Human Services, 2004). Suomi's (2004) research demonstrates that in non-human primates marked differences in maternal nurturing interact with genetic variations in certain neurotransmitters resulting in dramatically different outcomes for the offspring. This suggests mechanisms for human behavioral development, and potential avenues for targeted interventions in humans (Champoux et al., 2002; Suomi, 2004). The observation that certain parenting styles are associated with a young child's risk of being overweight creates important questions about identifying the mechanisms for this association and its interactions with genetic and other factors. Understanding these gene-social environment interactions is both a pressing need and an emergent opportunity (Tholin, Rasmussen, Tynelius, & Karlsson, 2005) that can best be addressed by the National Children's Study.

Psychosocial environmental influences appear to interact with physical and chemical exposures in complex ways. Environmental justice literature (Brulle & Pellow, 2006; Bullard, 1983; Bullard, 1990; Bullard & Wright, 1993) suggests that the impact of exposure to toxic substances may be greater in communities that have low levels of education and have poor access to health services. Social factors may also confound relations between physical exposures and health. For example, an association between exposure to an environmental toxicant and violent behavior may be misinterpreted as causal when, in fact, poverty causes the physical exposure and violence. Social factors and physical exposures may also modify or mediate the effects of one another on health outcomes. Sorting out these influences is essential if researchers are to understand why some children are healthy and thrive while others do not.

1.2.2.4 Biological Factors

A child's biologic environment ranges from in utero interaction with maternal physiology to nutritional, infectious, and allergenic exposures throughout childhood and adolescence. Accurate serial assessment of a child's multifaceted biologic exposures is important to understand the etiology and severity of NCS outcomes from preterm birth and congenital anomalies to insulin resistance and schizophrenia in adolescence.

Infection, inflammation, and stress

Maternal or early childhood exposure to many different organisms has been implicated in the subsequent development of outcomes to be studied in the NCS, including preterm birth (Andrews, Hauth, & Goldenberg, 2000; Goepfert et al., 2004; Pararas, Skevaki, & Kafetzis, 2006), neurodevelopment and psychiatric disorders (Hagberg & Mallard, 2005; Rapoport, Addington, Frangou, & Psych, 2005), and asthma (Garcia-Garcia et al., 2007; Sigurs et al., 2005). In contrast to the direct suppurative effects of infection, such as the cognitive and hearing losses associated with bacterial meningitis, the nature and timing of some putative associations suggests the distal influence of host inflammatory mediators produced in response to infection. However, the nature of the relation between in utero exposure to infection or inflammation and subsequent outcomes has been difficult to study in humans. For example,

the epidemiological literature suggests a strong association between maternal viral infection and subsequent schizophrenia in offspring (Bagalkote, Pang, & Jones, 2001; Yolken & Torrey, 1995). Animal studies demonstrate that in utero or early life exposure to circulating cytokines result in neuronal lesions compatible with schizophrenia (Gilmore, Jarskog, Vadlamudi, & Lauder, 2004; Meyer et al., 2006). Establishing a direct relation between prenatal inflammatory exposure and subsequent schizophrenia has been impossible because of time lags between exposure and outcome, which limit potential preventive and therapeutic strategies. The potential relation between in utero inflammation and autism has similar characteristics (Chauhan & Chauhan, 2006; Meyer et al., 2006), and the ability of the NCS to capture these early exposures offers similar opportunities for advancing etiologic understanding and prevention and treatment possibilities.

The relation between early infection or inflammation and asthma poses additional questions concerning the influence of immune response and subsequent disease. Numerous studies suggest that viral infection during infancy is associated with increased asthma risk (Garcia-Garcia et al., 2007; Sigurs et al., 2004). A related body of literature that is often presented under the rubric "hygiene hypothesis" suggests early exposure to infectious products, perhaps bacterial products in particular (Braun-Fahrlander et al., 2002), protects against subsequent development of asthma. Attempts to untangle this relation have focused on the impact of type and timing of infection on development of a strong Th-1 lymphocyte response as opposed to the persistence of Th-2 immunologic response associated with asthma and atopy (Effros & Nagaraj, 2007). Recent studies have suggested this is complicated even further by the timing of exposure to non-infectious allergens such as dust mite or animal dander (Holt & Sly, 2002).

An additional contributor to immune system development is exposure to maternal stress unrelated to infection and inflammation (Elenkov, 2004; von Hertzen, 2002). Genetic variation in the structure or activity of specific molecular mechanisms, particularly Toll-like receptors, also seems to influence the already complex relations (Vercelli, 2006). Serial measures of maternal and child infection and inflammatory response, emotional and physiologic stress, timing of exposure to a variety of potential antigens, and genomic analysis within the NCS will be integrated to enable an increased understanding of asthma etiology and the potential to develop new preventive and ameliorative strategies.

Elevated maternal glucose or diabetes

Compared to infants born to women without diabetes, infants born to women with a diagnosis of diabetes or other evidence of elevated blood glucose have an increased risk of congenital anomalies (Farrell, Neale, & Cundy, 2002; Guerin, Nisenbaum, & Ray, 2007; Nielsen et al., 2005; Schaefer et al., 1997; Sharpe, Chan, Haan, & Hiller, 2005). The amount of additional risk varies depending on the nature of the diabetes and the degree of maternal hyperglycemia. This may suggest a simple dose-response mechanism. However, a range of disparate major and minor defects with different embryologic origins is influenced by maternal hyperglycemia (Schaefer et al., 1997; Nielsen et al., 2005). Animal models suggest that one potential pathway through which maternal hyperglycemia disrupts normal embryologic development is via oxidative stress damage following increased fetal glucose metabolism (Loeken, 2006). The fetal oxidative stress response can influence selective dysregulation of individual gene expression and have differential effects on organogenesis depending on the timing and degree of maternal hyperglycemia. This mechanism may explain the similar effects of maternal hyperglycemia on the development of multiple and diverse organ systems. The potential role of oxidative stress in the etiology of at least some birth defects also dovetails with possible mechanisms of other exposures to be investigated in the NCS including infectious and inflammatory sequelae, diet, and respiratory pollutants.

Diet and nutrition

Aspects of maternal and child diets that are important to multiple outcomes within the NCS include overall caloric and macronutrient intake and potential exposure to pesticides or other chemical contaminants. Collection of additional dietary information will enable elucidation of potentially more subtle influences of diet on health and disease.

For example, in both human and animal diets with a high glycemic index and glycemic load, measures of a food's post-consumption impact on blood glucose (Frost & Dornhorst, 2005) have been associated with increased risk of obesity and type 2 diabetes which is independent of the diet's caloric content (Ludwig, 2002; Pawlak, Kushner, & Ludwig, 2004; Schulze et al., 2004). Further understanding of these presumptive relations is necessary if optimal interventions to curb the increase of obesity and related morbidity are to be developed.

A nutritional factor that may play an important modifying or protective role in relation to multiple NCS outcomes is dietary anti-oxidants. Oxidative stress has been hypothesized to play an etiologic role in numerous outcomes, including neurodevelopment and psychiatric conditions (Chauhan & Chauhan, 2006; Meyer et al, 2006); asthma (Effros & Nagaraj, 2007); birth defects (Loeken, 2006); and diabetes (Duncan & Ines Schmidt, 2006; Esposito et al., 2002). Evidence from human and animal studies regarding the ability of diets high in antioxidants to protect against disease is mixed (Abela, Howe, Oakes, & Webster, 2005; Devereux et al., 2006; Litonjua et al, 2006; Murray, Simpson, Kerry, Woodcock, & Custovic, 2006). The potential benefits of such a diet, however, retain biologic plausibility. For example, culture studies suggest the ability of antioxidants to prevent neuronal damage, although the developmental timing is crucial (Perry, Norman, Litzburg, & Gelbard, 2004). The longitudinal collection of systemic nutritional measures and dietary characteristics starting in utero and continuing through adolescence may help elucidate the role of oxidative stress in diseases and offer potential interventions.

1.2.2.5 Genetic Factors

The past decade has witnessed a virtual explosion in the development and application of genomic methodology and research that have direct application to the NCS. The majority of common disorders in children and adults are now recognized as having a "complex" multifactorial etiology, wherein multiple genetic and environmental factors play a role in disease causation (Kelada, Eaton, Wang, Rothman, & Khoury, 2003; Moore, 2003; Zondervan & Cardon, 2004). It is the interaction or multiplicative effects, rather than the sum of these factors, that likely underlies disease risk. These complex relations require that studies of disease causation assess each of these multiple factors in a common cohort of individuals as opposed to assessing different factors in different cohorts. In addition, changes in epigenetic factors and the association with environmental factors necessitate a longitudinal approach. This requires the kind of comprehensive assessment of genetic and environmental risk factors for disease in the same individuals and the large number of study participants to provide adequate statistical power (Garcia-Closas & Lubin, 1999) proposed in the National Children's Study.

The sequencing of the human genome provides powerful research tools to identify genetic variation that contributes to health outcomes (International Human Genome Sequencing Consortium, 2001; Venter et al., 2001). In the past, association studies using candidate genes have been the mainstay of epidemiologic investigations of the role of genetic and environmental factors in children's health. More recently, rapidly changing and increasingly affordable technology and information from the International HapMap Project have made whole genome association studies using haplotype tagging single nucleotide polymorphisms (SNPs) a reality (The International HapMap Consortium, 2005). The HapMap project has lessened the task of measuring millions of SNPs by using linkage disequilibrium to identify a reduced set

of "tag" SNPs for capturing variation throughout the genome (Johnson et al., 2001; Wall & Pritchard, 2005). As affordable technology becomes available, complete sequencing of the genome of NCS participants will be possible. Emerging systems biology approaches to genomic analyses, which seek to understand how different biologic systems are interconnected (Bogyo & Cravatt, 2007; Li & Burmeister, 2005) and how both the components and their relations can change over time, will benefit from repeated phenotypic and genomic measures in the NCS.

The Study will also have the power to examine gene-environment interactions from a developmental perspective in a new way. It will provide the opportunity to evaluate specific genetic factors in subgroups of mothers, fathers, and children in the Study. It will be a rich source of data that can be used to investigate the mechanisms behind complex diseases such as autism and asthma, the quantitative contribution of genetic variation to common conditions such as obesity, and the impact of gene and environment interactions on behavior and health outcomes. Multiple gene-environment and gene-gene interactions play a key role, creating the need for complex, computer-intensive forms of analysis. The analysis of genomic data is a field of much active research (Chatterjee, Kalaylioglu, Moslehi, Peters, & Wacholder, 2006; Heidema et al., 2006; Thornton-Wells, Moore, & Haines, 2004). Analysis of genotype effects, multi-locus genotype-genotype interactions (e.g., epistasis), and gene-environment interactions can be conceptualized in a regression analysis framework for different types of outcomes where the predictor variables include SNP genotypes, environmental exposures, epistasis (e.g., interactions) among SNPs, and SNP-environment interactions. Methods developed for analyzing high-dimensional data such as microarray gene expression, massively parallel signature sequencing (MPSS), and evolutionary trees of haplotypes may also be utilized. New analytic methods can be expected to emerge in the future and researchers analyzing the NCS genomic data will apply the best methods available in every phase of the process.

1.2.3 Conclusion

There is a well established vulnerability to the effects of environmental exposures for the embryo, fetus, infant, young child, and even the developing adolescent. There is a broad array of environmental exposures that have been identified as possible threats to children's health and development, only a few examples of which are noted above. Only for a small number of these exposures has empirical and theoretical evidence of their specific effects on children been established. Likewise, conditions and diseases in children that represent the major health threats of the new morbidity continue to challenge the researchers who seek to understand their genetic and environmental causes. The convergence of these experiences and scientific observations was a compelling rationale for the President's Task Force to recommend, and for Congress to direct, that NICHD conduct a longitudinal study of environmental influences (including physical, chemical, biological, and psychosocial) on children's health and development with a national scope, a large sample size, and a breadth of measures that are capable of identifying the environmental and genetic factors contributing to the major diseases and conditions that affect our children.

Chapter 2

Conceptual Design and Framework

2. CONCEPTUAL DESIGN AND FRAMEWORK

As dynamic and developing entities, children are exposed to an array of chemical, physical, and psychosocial environmental factors beginning in utero that affect their health, growth, and development and predispose them to later disease. Parental factors beginning prior to conception, and exposures in utero that continue through childhood, interact with the inherent genetic potential of the child to determine ultimate health. Most health outcomes are not the result of a single environmental exposure, an inherent genetic predisposition, or the interaction of a single environmental exposure and genetic factors. Rather, health outcomes are a complex amalgam of multiple environmental exposures over time that affect the inherent genetic makeup of each person.

2.1 Hypothesis Formulation and Study Design

The NCS is designed to respond to the significant challenge of delineating associations between single and multiple exposures over a long period of time, genetic factors, and health outcomes in children. The previous sections provide brief examples of hypothesized relations between various environmental exposures and major diseases or conditions of children, each having its own empirical and/or theoretical basis. To identify and confirm any one of these relations would best be accomplished with a prospective longitudinal study that incorporates the following criteria for design and data collection:

- Assessments of multiple exposures and multiple outcomes.
- Prospective and high quality data collection to decrease bias.
- Measurement of relatively rare outcomes (e.g., autism spectrum disorders, type 2 diabetes).
- Coverage of a long enough portion of the lifespan to measure and link early exposures with later outcomes.
- Repeated measures to capture the relation between exposures during critical time periods and trajectories of development.
- Generalizability to the U.S. population.

The NCS seeks to study the exposure-outcome relations for multiple exposures at earlier life stages and multiple outcomes related to the same or different exposures at later stages. This can only be accomplished through the use of a single large cohort with multiple exposure and outcome measures. This approach is more efficient and economical than multiple separate cohort studies and less biased than multiple retrospective case control studies.

The prospective cohort design is well suited to look at the multiple outcomes associated with a single exposure or set of exposures (Manolio, Bailey-Wilson, & Collins, 2006). For example, the NCS offers the opportunity to examine the potential impact of certain endocrine active compound exposures early in life on neurocognitive development and sexual maturation later in life (Cooper, Goldman, & Tyrey, 1998; Jahnke, Choksi, Moore, & Shelby, 2004; Landrigan, Kimmel, Correa, & Eskenazi, 2004;

Longnecker et al., 2003), thus linking these disparate outcomes to a single exposure or class of exposures and potentially identifying common underlying mechanisms.

Moreover, the breadth of measurements in the NCS will allow examination of the combined and independent contributions of multiple exposures on a single outcome. For example, exposure to certain pesticides, plasticizers, heavy metals, early life exposure to media, different parenting styles, and genetic predispositions are all hypothesized to have effects on cognitive development. Careful prospective collection of data across multiple exposure domains will allow assessments reflective of true exposure patterns of U.S. children. The general conceptual model for exposure-outcome relations in this large longitudinal study, including direct relations, mediated relations, and gene-environment and environment-environment interactions, is illustrated in Figure 2-1.

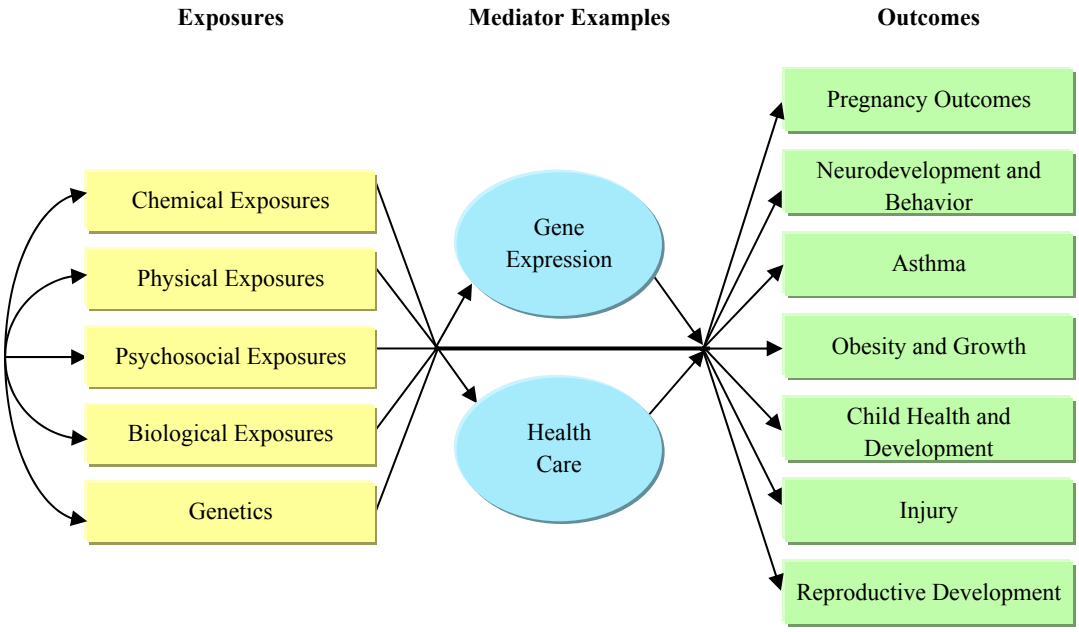

Figure 2-1. Conceptual Model of Exposures, Their Interactions, Examples of Mediators, and Outcomes

During the past several decades increasing numbers of reports have shown how different exposures interact with each other and/or with genetic factors to affect outcomes not seen with a single exposure or genetic variation alone. For example, the now classic study by Caspi (Caspi et al., 2002) replicated by Foley (Foley et al., 2004) demonstrated that maltreated children whose genotype conferred low levels of monoamine oxidase A (MAO A) expression more often developed conduct disorder, antisocial personality, and adult violent crime than children with a high-activity MAO A genotype. Similarly, Wang et al. (2002) reported that maternal CYP1A1 and GSTT1 genotypes modified the association between maternal smoking and infant birth weights. In another example, Berkowitz et al. (2004) found that mothers with both a low paraoxinase polymorphism and maternal elevation of metabolites of the pesticide chlorpyrophos had infants who had a small but significant reduction in head circumference. Other examples of environment-environment interactions include allergen-air pollutants (chemical-biological) with regard to induction of asthma (Diaz-Sanchez et al., 2006; Platts-Mills, Vaughan, Squillace, Woodfolk, & Sporik., 2001) and mercury exposure and socioeconomic

characteristics of child caregivers (chemical-psychosocial) in relation to cognitive development (Davidson, Myers, Shamlaye, Cox, & Wilding, 2004).

These findings from relatively small samples suggest causal relationships that require larger samples for replication and conclusive results. Analyses of gene-environment and environment-environment interactions require identifying subgroups and conducting subgroup analyses that are only possible with a large original sample. The sample sizes required for such analyses are described with specific hypotheses (see Appendix A-2), but, in general, samples approaching the proposed 100,000 are required. Unraveling the increasingly recognized complexities of environment-environment and gene-environment interactions offers great potential for targeted interventions but also requires increasingly complex and demanding subgroup analyses. Underlying this enormous potential is a requirement that these interacting factors must be measured in the same individuals who are followed prospectively for the outcomes of interest at later life stages. As long as the key measures for the various classes of exposure are obtained in the same individuals of the cohort, the opportunities exist to examine a variety of moderating processes, from how socioeconomic factors interact with chemical exposure (Agyeman, 2005; Anderton, Oakes, Fraser, & Anderson, 1994) to how parenting styles and maternal nurturing interact with specific neurotransmitter polymorphisms to affect major behavioral outcomes (Suomi, 2004; Champoux et al., 2002).

2.2 Significance of the Longitudinal Database as a Platform for Future Studies

As a longitudinal cohort study of considerable size and complexity, the NCS will provide answers to many current hypotheses. Because biologic and environmental samples and the extensive database will be stored and available in the future, however, it will also constitute a significant national database and resource to answer many questions not yet conceived.

2.2.1 Development of Future Hypotheses

A listing of the publications from the largest longitudinal study of child health, the Collaborative Perinatal Project (CPP) conducted during 1963 to 1989, reveals 611 separate publications. The topics and contributions of these publications vary widely from the intended original aim of the CPP, which was to identify the relation between neonatal asphyxia and cerebral palsy. With more extensive exposure data, a larger sample size, and a longer follow-up period than the CPP, the NCS provides even greater potential for opportunities to investigate questions regarding the health and development of children. This capacity for research and analyses beyond the stated aims of the Study constitutes a major contribution and impetus for undertaking such a project. Because of this potential, it is important to collect the samples and measurements in ways that optimize opportunities for future testing and analysis.

2.2.2 Health Disparities

The NCS will be able to address many major health disparities that currently exist in the United States, and to collect sufficient data to address others not yet recognized. The Children's Health Act of 2000 specifically directed that the NCS "consider health disparities among children ... ." Several major features of the NCS will allow the Study to address health disparities, to advance the state of knowledge about disparities, and to provide information that can guide policy and practice to reduce and eliminate disparities.

The representative, probability-based sampling approach ensures the Study sample will reflect the broad racial and ethnic diversity of the United States. The full NCS sample will consist of approximately 78,000 white, 19,000 Hispanic, 15,000 Black, 5,000 Asian, and 2,000 American Indian participants. Also, approximately 20 percent of the cohort will be from rural areas. This makes the NCS the most comprehensive, long-term study of this size for Hispanic children, Black children, and children from rural settings.

The size and diversity of the cohort will allow the NCS to generate large amounts of data and better characterize the disparities among subpopulations, including uncovering more subtle disparities than previously recognized. Disparities of interest to the NCS cut across a variety of the Study exposures and outcomes, from differences in the prevalence of preterm birth to variations in exposure to pesticides across communities to differences in the types of injuries children suffer.

Future researchers may find even more topics that prove timely and important. The wide range of study hypotheses that can be addressed using the NCS data also translate into a wide number of disparities that can be examined using the large data set. For many possible study questions, adequate power exists to perform analyses at the subgroup level.

2.2.3 Case-Control Studies

Many of the Study hypotheses can be addressed effectively and most efficiently through nested case-control studies. The prospective collection and careful storage of all Study data (biospecimens, environmental samples, stored images, etc.) will allow investigators to limit many of the expensive analyses to smaller subsets of identified cases and their matched controls.

Perhaps most importantly, this approach will allow the investigation of hypotheses formulated in the future. Some of the greatest values of this Study are the establishment of a databank of longitudinal measures and a repository of both environmental and biologic specimens that will allow future investigators to address important questions of clinical and public health relevance.

Chapter 3

Preliminary Studies

3. PRELIMINARY STUDIES

As part of the National Children's Study's conceptualization, the President's Task Force on Environmental Health Risks and Safety Risks sought advice concerning exposure measurement and study design from a panel of experts involved in recent or current major longitudinal studies. These included the Collaborative Perinatal Project, The Danish National Cohort Study, the Bogalusa Heart Study, The Avon Longitudinal Study, The Women's Health Initiative, The Framingham Heart Study, The Nurses Health Study, and HMO-based studies. In addition to strong endorsement and encouragement for a national longitudinal study of children's environmental health, the panel recommended the development of specific hypotheses that would frame the study and assure the most critical contemporaneous health concerns of children were not neglected. Additionally, the work group exhorted the planners to be bold and ambitious to ensure the study would be worth the considerable expenditure of time and resources (Iowa Department of Human Services, 2003).

3.1 Review of Existing Longitudinal Studies and Databases

Before the planning and initiation of a new large and expensive study proceeded, an inventory and review of longitudinal studies was commissioned by the National Center for Health Statistics and undertaken by the Lewin Group (2000). The review examined existing resources for assessing the possibility of addressing the Study goals without embarking on an entirely new study and identified needs for longitudinal research by the Centers for Disease Control and Prevention. This search sought to identify possible duplication of efforts by the proposed longitudinal cohort study. To identify virtually all of the significant longitudinal studies, two databases served as primary sources of identification: Medline and the listing of National Institutes of Health (NIH)-funded studies at the Community of Science, a network of scientists and research organizations on the Internet. Searchers used the terms "longitudinal studies," "cohort study," and "risk assessment." From more than 37,000 citations, the search identified 154 studies that met the criteria of longitudinal (studies must collect data at two points in time), longer than one year, prospective, observational (as opposed to interventional), general population (as opposed to disease specific), and meaningful sample size (generally 1,000 and greater) conducted in the United States. The Lewin inventory did not include studies that could be identified only through the behavioral, psychological, or social science literature or studies of occupational health. The studies from the initial search cover an array of health conditions in youth and adults, including, but not limited to, asthma, behavioral health, cancer, and child development.

A systematic review of all available longitudinal cohort studies found no study capable of answering the questions and concerns that led to proposed National Children's Study regarding potential long-term effects in children from environmental exposures. Although the Lewin inventory identified 62 longitudinal studies of youth and their health outcomes, only five met the criteria of a predominantly U.S. sample, sample recruitment during pregnancy or early infancy, and sufficiently large sample size (greater than 10,000). Of these five, only one, the Early Childhood Longitudinal Study (ECLS-B) Birth Cohort (National Center for Education Statistics, 2000) met the above criteria and could possibly be adapted or used as a framework for a large longitudinal cohort study of environmental factors and children's health. The goal of the ECLS-B is to assess the health, growth, and developmental factors critical for school readiness and achievement. It identified a nationally representative sample of approximately 15,000 children at birth and is performing examination batteries at 9, 18, 30, and 48 months of age. Because the ECLS-B recruited participants at birth, the issues involved in recruiting during the prepregnancy or early pregnancy period still needed to be identified. Thus, the ECLS-B excluded the possibility of observing effects of prenatal and infancy exposure and did not collect data for any chemical or biological exposures.

Ongoing population-based studies of the National Center of Health Statistics were also considered as resources to address concerns about environmental effects in children. These included the National Health and Nutrition Examination Survey (NHANES), the National Survey of Family Growth (NSFG), the National Maternal and Infant Health Survey (NMIHS), the National Health Interview Survey (NHIS), and vital statistics. Of those surveys, only NHANES met key criteria of activity that is done on a continuous or relatively frequent interval, and of the ability to collect physical measurements of the child or environment, or biomarkers, in the context of the effort. While NHANES serves extremely important surveillance and monitoring functions, it is not a cohort study and its cross-sectional design does not permit it to identify the kinds of exposure-outcome relations critical to the goals of the NCS. NHANES collects data on approximately 5,000 people per year selected to be a nationally representative sample of the U.S. population of all ages (Centers for Disease Control and Prevention, National Center for Health Statistics, 2007). In the course of this effort, mobile examination centers (MEC) and technical personnel travel around the country collecting the data. Since NHANES is representative of all ages, the numbers of children are relatively few overall, and it would take many years to gather information on the number of children required for the work proposed on the NCS. The importance and uniqueness of the proposed Study is its ability to examine exposures very early in development, including intrauterine exposures. Given its household sampling frame, NHANES would contain too few pregnant women to enable detailed analysis. Most importantly, NHANES is not designed to do multiple assessments in specific individuals over time.

3.2 NCS Planning and Methods-Development Studies

More than 2,500 scientists and other professionals had input on the NCS. Guided by the Interagency Coordinating Committee (ICC) of scientists and staff of the federal funding agencies (HHS, NICHD, NIEHS, CDC, EPA) a federally chartered advisory committee (NCSAC) was established under the Federal Advisory Committee Act. The NCSAC established 22 Working Groups, comprised of federal and non-federal scientists, that focused on specific scientific areas or aspects of the study (see Appendix J for a list of working groups). Most of the Working Groups focused primarily on defining the domain-related hypotheses (see Chapters 4 and 7 and Appendix A for details of hypotheses) and study methods that were subsequently reviewed by the NCSAC and incorporated by the ICC as the Study core hypotheses.

The Study planners used a range of approaches to address the numerous issues and questions they faced, including large conferences, workshops, scientific reviews or "white papers," and actual methods-development studies that were labeled "pilot studies." Five large assemblies were held to exchange information and science related to the Study and to provide venues for the Advisory Committee and Working Groups to conduct activities. Thirty-one extremely useful workshops with subject-matter experts have been conducted thus far to define and to clarify scientific issues and methods that could be applied to the various constructs of interest to the Study. For example, workshops on dietary assessment and on the collection and use of genetic information helped identify measurements and assays applicable to the Study and eliminate inappropriate or unfeasible measures. Along with reports from the respective Working Groups, the workshop proceedings were used for input and as a starting place for specific protocol planning. Reports from the workshops are posted on the Study's Web site: www.nationalchildrensstudy.gov.

For a number of aspects of the Study, more detailed reviews and analyses of the scientific literature were needed to inform decision making, and literature reviews or white papers were also commissioned to provide essential guidance for a number of critical issues. For example, to decide the Study's sampling strategy, a series of papers was commissioned to review topics including alternative sampling strategies; the impact of anticipated recruitment and retention rates on sampling options; the

impact of sampling options on core hypotheses; and cost estimates for sampling options. In addition, a series of methods development or pilot studies was conducted to answer specific questions or to develop specific methods. Such studies varied in methods and objectives. For example, focus groups were conducted, using a variety of sources (i.e., young mothers, adolescents, health providers), regarding attitudes and perceptions related to the NCS. One study evaluated the feasibility of three-dimensional versus two-dimensional ultrasound for measurement of fetal growth, which led to the more economical decision to use two-dimensional ultrasound. Another study, testing the feasibility of employing clinical practice sites for data collection venues in the NCS, identified a number of important issues to be addressed where this strategy is used. Thirty-five white papers and 29 pilot or methods development studies have been conducted thus far, and reports are available on the NCS' Web site. Many of these projects have applicability beyond the NCS and have been published in the scientific literature. Lists of the workshops, white papers, methods-development studies, and publications that have come out of these projects appear in Appendix J.

Chapter 4

Aims and Hypotheses

4. AIMS AND HYPOTHESES

4.1 Specific Study Aims

The National Children's Study has several broad aims. These aims will be served through a program of current, carefully designed research questions and the creation of a resource for future research questions. The specific aims of the Study are:

(1) Determine the presence or absence of effects, both harmful and helpful, related to the timing, frequency, magnitude, and duration of specific chemical, physical, biological, and psychosocial exposures in children's environments from preconception to adulthood.

(2) Determine possible environmental contributions to, or causes of, specific diseases and conditions of children, including, but not limited to, prematurity and other outcomes of pregnancy, neurological and developmental disorders, psychiatric and behavioral disorders, altered physical development and sexual maturation, obesity and insulin resistance, asthma, and injuries.

(3) Determine how genotypic variation and mechanisms, and the interaction of genes with environmental factors, influence disease risk and developmental trajectories in children.

(4) Serve as a national resource for future studies of child health and development by providing a rich database and repository of environmental and biological samples and information that can be used to address future questions and hypotheses.

4.2 Core Hypotheses

The rationale for a large longitudinal study with multiple classes of exposure, outcome, and genetic measures to address the Study aims has been described. These aims are the sum of specific hypotheses examining how environmental and genetic factors may affect children's health and development. Thus, the NCS can be can best be understood as a broad program of research comprising multiple separate and overlapping hypothesis-driven studies, each requiring this proposed design and size.

The NCS planners recognize that framing hypotheses is essential to guide study planning and to assure that important questions can be addressed. Nonetheless, not all important or answerable questions are necessary or even possible to state. However, in planning the Study a standard was established that a supporting hypothesis must be required for inclusion of measures or design elements in the Study. Within broad priority exposure and outcome areas, the NCS has framed 26 well-defined core hypotheses to fulfill its aims to ascertain whether exposures to environmental factors either adversely or positively affect the health and development of children and whether certain health conditions of children result from environmental exposures.

To derive the core study hypotheses, the NCS relied on the expertise and input of a Federal advisory committee (National Children's Study Advisory Committee [NCSAC]), its working groups, and the general public. Within priority areas, many hypotheses were proposed by working groups and other entities and then considered by the NCSAC, which made recommendations concerning their relevance

and prioritization. Ultimately, the NCS Interagency Coordinating Committee[1] established the core Study hypotheses (see Table 4-1). A more detailed listing by priority outcome area appears in Appendix A-1. Fully documented and referenced hypotheses across different priority areas are found in Appendix A-2. These hypotheses identify relevant environmental exposures including physical, chemical, biologic, and psychosocial factors that affect the identified priority outcomes, including pregnancy outcomes, neurodevelopment and behavior, injury, asthma, obesity and growth, child health and development, and reproductive development. Many hypotheses also take into consideration the vital impact of gene-environment interactions or the effect of access to health care services on health and well-being. The potential avenues of investigation are too numerous to cite, but a number of specific study questions have been developed to assure that key measures are obtained, and that the sample of participants and the study design are adequate to address the questions. Acknowledging that science evolves, this list of hypotheses is expected to change as additional existing hypotheses are refined, omissions of important questions are identified, and other hypotheses become outdated.

[1] The Interagency Coordinating Committee is comprised of senior Federal staff scientists assigned since 2000 to lead the development of the NCS on behalf of the lead Agencies supporting the NCS: the Department of Health and Human Services, National Institute of Child Health and Human Development, National Institute of Environmental Health Sciences, Centers for Disease Control and Prevention, and the U.S. Environmental Protection Agency.

Table 4-1. Hypothesis Topics of the National Children's Study

- Birth defects from impaired glucose metabolism
- Increased risk of preterm birth from intrauterine exposure to mediators of inflammation
- Increased risk of fetal growth restriction, preterm birth, birth defects and developmental disabilities in children born through assisted reproductive technologies
- Maternal subclinical hypothyroidism and neurodevelopmental disabilities/adverse pregnancy outcomes
- Non-persistent pesticides and poor neurobehavioral and cognitive skills
- Prenatal infection and neurodevelopmental disabilities
- Gene-environment interactions and behavior
- Prenatal and perinatal infection and schizophrenia
- Family influences on child health and development
- Impact of neighborhood and communities on child health
- Impact of media exposure on child health and development
- Social institutions and child health and development
- The role of prenatal maternal stress and genetics in childhood asthma
- Exposure to indoor and outdoor air pollution, aeroallergens, and asthma risk
- Dietary antioxidants and asthma risk
- Social environmental influences on asthma disparities
- Early exposure to structural components and products of microorganisms decreases the risk of asthma
- Obesity and insulin resistance from impaired maternal glucose metabolism
- Obesity and insulin resistance from intrauterine growth restriction
- Breastfeeding associated with lower rates of obesity and lower risk of insulin resistance
- Fiber, whole grains, high glycemic index and obesity and insulin resistance
- Genetics, environmental exposures, and type 1 diabetes
- Repeated mild traumatic brain injury and neurocognitive development
- Behavioral exposures, genetics, and childhood or adolescence onset aggression
- Antecedents and resiliency to traumatic life events in childhood
- Hormonally active environmental agents and reproductive development

PART II
STUDY DESIGN AND METHODS

Chapter 5

Challenges and Approaches

PART II: STUDY DESIGN AND METHODS

5. CHALLENGES AND APPROACHES

5.1 Design Challenges and Approaches

A major challenge in designing the National Children's Study is to balance the power of the large sample size, enrollment of women early in (or prior to) pregnancy, longitudinal follow-up, and breadth of exposure and outcome measurements with the real life considerations of participant burden and cost. Because the Study is observational, respondent burden must be commensurate with the motivations and limited benefits of participation. Using other longitudinal observational studies (Golding, 2004) as a guideline, the NCS will try to limit any single face-to-face data collection to no more than half a day (four hours). Similarly, procedures must pose no more than minimal risk, and biospecimens will be limited in quantity. Thus, only some of the many possible clinical assessments are appropriate, and only a finite number of tests can be run on each specimen. Funds are also limited, thus the planned tests or procedures implemented in the entire cohort must be carefully selected. A core set of contacts and data collections with Study participants has been proposed, and forms the framework for the Study. It is expected that sub-studies and adjunct studies will supplement the full Study, utilizing additional, more in-depth data collections on targeted areas of interest and involving subsets of the full Study population (see Chapter 16).

5.2 Measurement Challenges and Approaches

A number of approaches will address the measurement challenges of this large, longitudinal, epidemiological study. The best measures possible will be employed in the full Study, but they may not always mirror more in-depth assessments used in more circumscribed research studies because of the cost, time, and burden involved. Again, it is expected that these issues can be addressed in sub-studies or adjunct studies. For some measures, for example certain of the unstable and expensive environmental chemical assays that are important to assess in the entire population, a validation subsampling methodology will be considered to reduce costs while still including critical exposure measures (Strauss, Lehman, Morara, & Ryan, 2003). It is likely that more extensive measures focused on specific areas will be utilized in anticipated sub-studies or adjunct studies.

To limit respondent burden, questionnaires developed for use in the core protocol incorporate short versions of scales or selected targeted subscales, when possible and appropriate. Moreover, a number of the questions are viewed as "screens" and will be followed by a lengthier set of questions for those with a positive screen. Face-to-face data collections are supplemented with mail-in or computer-based self-administered data questionnaires. These additional remote data collections not only provide valuable data for the Study, but also serve as a means to maintain meaningful, frequent contacts with Study participants.

5.3 Processes for Continuing Protocol Development

As a longitudinal study of more than 20 years, the NCS will face demands of continually changing and advancing science, technology, and methods. Wherever possible, the Study must anticipate and accommodate evolving science and technologies. For example, a large National Institutes of Health (NIH) program to develop efficient, inexpensive, and accurate exposure assessments is underway. It is

likely that some of the methods from this initiative would increase the power of environmental assessments for the NCS (http://www.gei.nih.gov/exposurebiology/index.asp). Development of technologies and methodologies for genomic measurement and analyses will continue to evolve rapidly during the Study. It is expected that as this field advances and expands, so too will the opportunities for understanding gene-environment relations and mechanisms of disease. Information management systems (IMS) can be expected to be outdated at least every 5 years, and the IMS for the NCS is being built to allow incorporation of changes as they occur.

Accordingly, the Study's protocol will require continual planning for each successive phase of the cohort as the participants advance through life. The initial research plan and protocol focus on the first phase of pregnancy and on infancy. Subsequent plans and phases will address preschool, elementary school, early adolescence, and late adolescence. Planning the specific methods for these respective protocols will be undertaken as the cohort approaches each phase with enough time for careful planning and implementation but close enough to the needed protocol so methods can be as current as possible.

As in the planning of the Study thus far, future protocol planning will seek to include the best scientific input possible. To seek input and guidance on specific issues, The Study will continue to utilize the Federal Advisory Committee, ad hoc workshops, and literature reviews and white papers. When needed, methods development and pilot studies will be conducted to resolve issues and to refine measures for each respective phase. In contrast to the first several years of Study planning, the Study will not have numerous expert working groups under the Advisory Committee. Instead, investigators from the 30-40 Study Centers and Coordinating Center will provide ample depth and breadth of expertise in the form of subject matter expert teams for input into planning the respective protocols. These working teams will propose methods and measures for the respective protocols to the staff of the Program Office and Coordinating Center. The Steering Committee of Principal Investigators will consider and propose decisions regarding Study priorities and similar issues, and the Interagency Coordination Committee will continue to provide review and oversight with regard to the Study's ability to address the goals and missions of the respective federal agencies. Though committed to input from broad scientific expertise, ultimate decision-making authority rests with the Director of the National Institute of Child Health and Human Development and, on his behalf, the Director of the NCS.

Chapter 6

Sample Design

6. STUDY DESIGN

6.1 Overview of Study Population

The National Children's Study is a longitudinal study that will enroll and follow over time a nationally representative sample of approximately 100,000 children born in the United States. Participation in the Study is voluntary, so potential Study participants can choose not to participate at any time. The Study calls for collecting information on children from birth through age 21. However, to enable assessments of exposures and risk factors during critical periods of embryonic and fetal development, some information must be collected from women before they become pregnant, and additional information must be collected while the woman is pregnant. Thus the Study plan is to select a sample of women of child-bearing age, to request that each eligible woman participate in the Study, and to follow these women over a fixed period of time. If an eligible woman informs the Study that she is planning on becoming pregnant or she is "at risk" of becoming pregnant, certain information will be collected on preconception exposures and risk factors. If any eligible woman in the selected sample becomes pregnant, information will be collected during her pregnancy. The collection of mother's information will begin as early in the pregnancy as possible and, at the time of birth, her child will be enrolled in the sample of children. It is recognized that not all pregnancies will result in a live birth; the Study protocol addresses human subject concerns and issues related to adverse pregnancy outcomes.

Thus, for the NCS, children/births are sampled through the mothers. Because not all pregnancies are planned, not all mothers will have preconception information collected. The Study target is to enroll at least 25 percent of pregnancies prior to conception and to identify and enroll a cumulative total of 90 percent of pregnancies before the end of the first trimester of the pregnancy. For most Study locations, births will be enrolled during a 4-year period with a target of 250 births per Study location per year. As described below, there are 105 Study locations in the national design. Most locations correspond to a single county, but some are comprised of multiple counties.

6.2 Inclusion and Exclusion Criteria

The sample design described below calls for recruiting women into the Study primarily through household sampling. All women who are in the first trimester of pregnancy at the time of initial contact with the Study are eligible for inclusion. Additionally, women between ages 18 and 44 at the time of initial contact who are not pregnant are eligible for enrollment and follow-up for pregnancy. If at any time in the enrollment period it is determined that a particular woman cannot become pregnant, she will not be followed. The frequency and intensity of follow-up of women who are not pregnant depends on the woman's probability of becoming pregnant as described in Section 6.4.2.

If a woman enrolled in the Study gives birth during the 4-year enrollment period, the newborn is included in the Study provided the mother resides in a household that is part of the Study sample at the time of the delivery. All births to mothers who meet the initial eligibility criteria are eligible for Study enrollment, including children born to surrogate mothers, those expected to be adopted or assigned to foster homes, and births to women who are on active duty in the military.

Women who are cognitively impaired or mentally ill are not eligible if they are not able to understand fully the Study requirements and grant informed consent.

6.3 Sampling Strategy

A number of study and sampling design options were considered for the NCS (see Sample Design Options and other related documents available at http://www.nationalchildrensstudy.gov/events/advisory_committee/other_work_062004.cfm). There are advantages and disadvantages to each of the candidate approaches, however, after careful consideration and upon the advice of the NCSAC, a national probability sample of all U.S. births was chosen as the design that best fulfills the following goals:

- Collection of high quality, objective data to minimize measurement biases
- Avoidance of selection biases and other biases that could lead to invalid inferences concerning exposure/outcome relations
- Ability to capture the diversity of the U.S. population such that both the range and diversity of exposures and outcomes are represented
- Ability to generalize results of the NCS to the U.S. population

The sample design for the NCS is a multistage probability sample of births in the United States where the births are identified from a sample of households. The design includes two or three stages of sampling.

The first stage of sampling was the selection of primary sampling units (PSUs), which correspond to single counties or groups of contiguous counties. The second stage is the selection of smaller geographic areas (segments) from within the primary sampling unit. In general, these segments comprise city or suburban blocks or combinations of blocks and roughly correspond to neighborhoods. The third stage, which applies only to very densely populated segments, involves the selection of groups of households from within the segments. Each stage is detailed below.

6.3.1 Selecting Study Locations

The process for selection of Study locations was based on the need to achieve representative coverage of the United States with respect to geographic areas, metropolitan/nonmetropolitan areas, and demography. All decisions on sample design options considered costs, coverage, statistical reliability, and practical concerns of the protocol. Cost models and logistical aspects of the NCS data collection led to the design decision to use 105 study locations.

The probability of a county being selected as a PSU is based on the number of births to residents of that county. Because the number of births in a county at a future date cannot be known, data on resident births (births based on the mother's residency at the time of birth) from four recent years (1999-2002, the most recent four-year period available at the time) were used as an estimated measure of size for sampling the PSUs.

The 3,141 U.S. counties were categorized into 18 large strata defined by metropolitan status (metro, nonmetro) and geography (nine census divisions). Within each of the 18 large strata, the total number of births determined the initial number of smaller strata. Based on their number of births, 13 counties were large enough to be designated as self-representing units (also referred to as certainty units). For three of these counties, the number of births was so large that each county was assigned multiple PSUs. Los Angeles County was assigned four PSUs, Cook County, IL, (containing Chicago) was

assigned two, and Harris County, TX, (containing Houston) was assigned two. These are units that were "certain" to be selected into the probability sample based on their large number of births. Thus, the design contains 13 locations but 18 PSUs that are considered self-representing.

The remaining 3,128 counties were placed into smaller strata. Within each of the 18 large strata, these smaller strata were formed to be of roughly equal size. The smaller strata were defined in terms of the size of county or the percent of births with specific characteristics. The characteristics used to define the smaller strata were percent of births to Native American women, percent births to Asian women, percent births to Hispanic women, percent births to Black women, and percent low birth weight. After all strata had been formed, one PSU per strata was selected with a probability proportional to size (i.e., number of births).

A minimum measure of size for a PSU was established as 2,000 births during a 4-year period (or an average of 500 births per year). If a county was selected that had fewer than 500 births per year, geographically adjacent counties in the same stratum were added until the PSU met the minimum measure of size. In a few cases, that criterion could not be achieved. For such cases, an additional PSU was selected.

The final first stage sample comprised 110 PSUs in 105 locations: 26 locations are non-self-representing PSUs from nonmetropolitan strata; 66 locations are non-self-representing PSUs from metropolitan strata; and 13 locations with 18 PSUs are from self-representing metropolitan strata. While this design is generally consistent with an equal probability sample design, differences in the sizes of the strata relative to the PSU probability of selection results in some variation. See Appendix B for a map of study locations.

6.3.2 Sampling within Locations (PSUs)

To meet the analytic needs of the Study, a total sample size of 1,000 enrolled live births is the target for each sampled PSU. With an enrollment period of 4 years, a sample size of 250 enrolled live births per year in each PSU is needed. (The Vanguard Centers have an additional year of enrollment and thus have 1,250 targeted births.) Because each selected PSU has greater than 250 births expected per year, a sample of births within each PSU must be designed and selected. This leads to the second stage of selection for the NCS. It is not feasible to take a simple random sample of births within each PSU. The second stage of the NCS design consists of forming small geographic units within a PSU called segments (or secondary sampling units) and then selecting a sample of those segments for inclusion into the Study.

6.3.3 Segment Sampling

To increase the operational efficiency, reduce costs, and provide for more useful representation of neighborhood-level characteristics, the segments within the PSUs are "clusters" of households. A geographic classification used by the U.S. Census Bureau (blocks nested with block groups, block groups nested within census tracts) is used to form segments. An advantage of using census geography is that data from other sources for these units can be linked to the sampled segments.

Prior to the formation of segments in a PSU, a target number of sampled segments is established. This number is primarily based on operational considerations and varies between PSUs. For most PSUs, it is expected that the number of sampled segments will be between 10 and 15. In general, a smaller number of segments are targeted in more rural, less densely populated PSUs that cover large areas; in more densely populated PSUs with larger numbers of births, the number of sampled segments

may be larger. The segments are constructed to be as uniform in size as possible within a PSU, but slight departures from the target segment size are expected.

As was done for the selection of PSUs, segments will be stratified to improve the precision of estimates and to ensure the sample is representative with respect to the stratum definitions. The NCS segments will be formed by combining a number of census blocks or block groups. Stratification can be done either before or after segments are formed. When stratification is done beforehand, the characteristics of the block groups can be used to form strata and only block groups in the same strata are then combined to form segments. These segments are homogenous with respect to the stratification variables but may not be geographically contiguous, thus increasing data collection costs. When stratification is done afterward, contiguous block groups can first be clustered to form segments and then "similar" segments are grouped to form strata.

It is expected that the segment stratification scheme will vary from PSU to PSU, with a goal of achieving locally defined neighborhoods as segments. (It is hoped that using locally defined neighborhoods will increase study participation rates and facility data collections at the community level.) Within most PSUs, geographic stratification will be used either as the sole stratifying variable or in combination with other variables. Geographic stratification is useful because many of the characteristics that differentiate subpopulations (such as income, race/ethnicity, educational attainment, and environmental measures) tend to be geographically clustered.

The strata are formed as equal in size as possible so that with approximately equal-sized segments, an approximately equal probability sample of segments is obtained. In some cases, it is desirable to allow for some variations in stratum sizes within a PSU to construct more homogenous strata than an equal-sized-strata scheme would permit. If the strata vary in size within a given PSU, the segments also vary in size across strata to equalize the sampling fraction within each stratum. For example, if one stratum is twice as large as another stratum within a given PSU, the segments within the first stratum are constructed to be twice as large as the segments within the second stratum.

In some cases, the strata are not geographically contiguous. This is typically the case when variables other than geography are used for segment stratification. In these cases it is necessary that each disjointed part of a stratum be large enough to form complete segments with minimal variation in segment size.

One challenge in having PSUs that have different sizes (number of births) is the large variation in the number of possible segments across PSUs. For example, among the Vanguard Centers, the smallest PSU has only 11 segments whereas the largest has approximately 1,800. A large number of segments causes difficulties in both forming and reviewing segments. In order to use resources more efficiently, a three-stage sampling protocol is used for large PSUs (typically those with more than 500 segments).

In large PSUs, geographic units are formed within strata and these geographic units, which vary in the total number of estimated births, are sampled with the probability of selection proportionate to the size of the geographic unit. Within each stratum, exactly one geographic unit is selected. Segments are then formed within the sampled geographic unit to be equal in size. Across strata, the segments are made equal in size if the strata are equal sized, or vary in size proportionate to the variation in stratum sizes if the strata are not equal sized. Within each sampled geographic unit, exactly one segment is randomly selected.

6.3.4 Listing and Enrollment

In selected segments, household screening is attempted in all households (dwelling units [DUs]) in the segment. The exception is a very large segment, which cannot be subdivided during segment formation. In such segments, DUs are subsampled. If one of these large segments is selected, the segment is divided into "chunks" and then a chunk is randomly sampled for listing and enrollment. For example, suppose a given segment is twice as large as the target segment size and consists of two very large apartment buildings that contain approximately equal numbers of DUs. In that case, each apartment building is a chunk, and one of the two is randomly selected to be retained in the sample. Other approaches for chunking (depending on the situation) include using floors of apartment buildings or block faces as chunks.

Household screening is attempted in each sampled DU, and all eligible women are enrolled. The scheduled monitoring of eligible women is dependent on each woman's likelihood of becoming pregnant. Women more likely to become pregnant are contacted more frequently (see Section 6.4.2). In some instances, the composition of the household will change or the DU will have new occupants. To enroll births from mothers in these situations, all DUs will be contacted at least once a year. This contact will be used to update the status of enrolled women's likelihood of pregnancy and thus her schedule for follow-up visits.

6.3.5 Rollout of PSUs

A sample of seven PSUs was selected to serve as the Vanguard Centers. These seven Vanguard Centers will serve as a platform to develop methodologies and procedures that will be refined and implemented throughout the Study. The remaining 98 PSUs will be introduced in three waves. The specific plan for the subsampling of the PSUs into the waves is currently under consideration. Pilot data collection is planned to begin in the Vanguard Centers in mid-2008, data collection in the first wave of additional PSUs is planned to begin in mid-2009 with the second wave two years later and the final wave two years after that.

The 98 PSUs not covered by the Vanguard Centers will be covered in the subsequent waves by the addition of Study Centers. Each Study Center will oversee participant recruitment and data collection at one to three geographically proximal study locations. The Vanguard Centers and Study Centers will work with the NCS Coordinating Center and the NCS Program Office to ensure effective development and implementation of study procedures.

6.3.6 Subsamples

In addition to the core set of measurements collected from all study participants, a number of data collections are being considered that involve collection of survey information, samples, or biological specimens from a subset of the total population or only at the community level. One example would be to reduce the proportion of samples obtained with nonmeasurable concentrations of an environmental substance. Questionnaire information on recent pesticide applications could be used to determine what homes will have air samples collected for nonpersistent pesticides since the air concentrations of these chemicals tend to decrease over time. Pesticide measurements in drinking water are currently being planned only in rural areas for homes using private wells since municipal water system information would be available for other locations and pesticide concentrations in drinking water in urban areas are often below detection limits. In some cases, environmental samples will be collected but not analyzed (e.g., metals in dust) unless biomarker concentrations (e.g., blood levels) indicate higher exposures have

occurred, and there is a need to determine the media or sources contributing to this exposure. Additionally, the large sample size of the National Children's Study affords the opportunity for more in-depth studies of subsamples within the framework of the longitudinal cohort study. A mechanism for adjunct study proposals is described in Chapter 16. Finally, to optimize the study's ability to incorporate state-of-the-art measurements, including some too costly or too burdensome for implementation in a sample of 100,000, the use of a validation sampling approach might be considered for certain measures. In this approach, a simple or less costly assessment is paired with the more costly or burdensome approach in a planned subsample of the population. For example, personal monitoring may be the best way to measure direct exposure to air pollutants or pesticides, but the cost and intrusiveness of this monitoring make this impractical to use on the entire cohort. The relation between the two assessments of the same domain is used to characterize and adjust for "measurement error" in the analysis of exposure-outcome relations for the entire cohort, although the majority of the study participants receive only the simpler, less expensive assessment. Similarly, a matrix approach for other applications (e.g. varying times of assessment) is also being considered.

6.4 Participant Recruitment

6.4.1 Recruitment Goals

The goal of recruitment is to obtain the highest response rate possible to reduce the potential for nonresponse bias. The minimum goal for combined response and coverage in each location will be between 65-75 percent. Study locations with traditionally lower survey participation rates will have lower targets. For example, in highly urban areas response rates for surveys are often considerably lower than in other settings.

To assess the impact of nonresponse bias, studies will be undertaken to assess the differences between responders and nonresponders. Lower response rates are acceptable only if it can be demonstrated that the nonrespondents are missing at random, or if a nonresponse assessment provides an adequate statistical procedure to adjust NCS estimates for nonrandom missingness. This combination of rigorously conducting the Study to obtain response rates as high as feasible along with studying the characteristics of nonrespondents is consistent with new standards and guidelines developed and distributed by the Office of Management and Budget.

6.4.2 Enumeration of Households

Within selected segments, all households will be enumerated to identify women of child-bearing age living in the household. This enumeration will be conducted in person by trained interviewers using computer-assisted personal interviewing techniques. An adult household reporter (age 18 or older) will be asked to answer questions about the number of household members, the number of males and females, and for females, their ages and their relationships to the household reporter. To ensure coverage of all dwelling units within each structure, questions will also be asked about other dwelling units that may not be easily visible or obvious, and therefore may have been missed during the listing process.

Two groups of age-eligible women (18-44) are targeted for enrollment: women who are in their first trimester or pregnancy, and women who are at some probability of becoming pregnant during the four-year enrollment period. After the age-eligible women are identified from the household enumeration, a separate pregnancy screener will be completed with each woman to determine her status. This will be done using a standardized set of questions related to her age, history of prior births, contraceptive use, and sexual activity. To ensure privacy these questions the pregnancy screener will be

administered in-person using computer-assisted self-interviewing techniques, which allow the woman to enter her responses directly into the computer. An audio feature of this will be included to read the questions to the woman to further ensure privacy and to circumvent possible literacy issues.

Women who are not currently pregnant, and who are not actively trying to become pregnant, or who are trying to become pregnant but based on the pregnancy screening have a relatively low probability of becoming pregnant, will be categorized as either "low probability" or "moderate probability." These groups will receive periodic phone contacts to determine if they have either become pregnant or, based on a limited set of screening questions, have moved to the group at higher probability of pregnancy. Women who are at high probability of becoming pregnant will be enrolled in the preconception cohort and actively followed for four menstrual cycles following enrollment. It is estimated that 55.2 percent of women in this group will become pregnant during this timeframe.

There will be periodic rescreening of households in selected segments to monitor for "move-ins" and other changes in the composition of the household living at each address. This periodic rescreening will take place only for those households where no eligible women are identified (estimated to be approximately 70 percent of all households). For those households with women being followed as part of the Study, scheduled contacts will be used to update information about household membership. This will be an important mechanism for monitoring changes in household composition as well as for identifying young women who "age in" (i.e., turn 18) during the four-year enrollment period.

6.4.3 Recruitment through Prenatal Care and Other Mechanisms

The primary mechanism for recruiting women for the Study is by contacting them in their households and encouraging them to participate in all phases of the Study. Some women, however, will move into sampled segments after the segments have been screened (and prior to the recontacts discussed above). Since children born to women living in the sampled segments are eligible, other mechanisms are needed to identify and recruit these women.

A supplemental mechanism to recruit eligible women (those living in the sampled segments) is through providers of prenatal care, birthing centers, and hospitals. All of the requirements of those sampled in households must be satisfied by these women, so this is simply another technique for identifying and recruiting eligible women from sampled households. In addition to increasing the Study's ability to cover the mobile population that otherwise would be missed, this supplemental recruitment also provides another opportunity to encourage participation from women who previously chose not to participate in the Study when contacted in the household screening. While this method is useful in reducing nonresponse and undercoverage, it does not provide full data from the pre-pregnancy and early pregnancy data collections and is thus viewed as a supplemental approach.

6.5 Community Outreach and Engagement

The NCS values community engagement, but it will not follow a strict community-based participatory research model. Community-based participatory research is defined as a collaborative research approach designed to ensure and organize participation in all aspects of the research process and action, emphasizing participation by the communities affected by the issue being studied, by representatives of organizations, and by researchers. Because the protocol includes data collection from multiple study sites to answer specific study questions that require a national sample, it was not possible to define the core study questions and initial protocol development through input of local communities or to account for their varied needs. However, principles of community-based research will be applied when

feasible and appropriate. A partnership with each community will be formed to ensure mutual respect and the establishment of an enduring relationship. Genuine community engagement offers the hope of enhancing recruitment, retention, and participant satisfaction.

Since the beginning of planning, the NCS has undertaken a range of community engagement activities to lay the groundwork for Study Center activities. Between 2000 and 2005, the NCS conducted many focus groups to obtain community perspectives on informing communities about the NCS, gaining the support of communities, recruiting and retaining participants, and NCS sampling and visits. Additionally, the establishment of working groups, the Study Assembly, and the Federal Advisory Committee allowed ongoing community input into the Study plans. The Vanguard Centers are working within local communities to prepare for recruitment. Study Centers will continually share experiences with and learn from each other in implementing community engagement plans.

Ideally, Study Centers will be able to build upon prior local community networks and relationships. However, the unique sampling strategy, data collection intensity, and length of the NCS necessitate different approaches to working with communities than previous studies or projects. To build trust, enhance the credibility of the Study, and ensure community engagement on the local level, during the first year of the Study the investigators from the Centers will conduct community needs assessments to identify children's environmental health issues in the target community. These assessments will focus on community concerns regarding the core NCS protocol and additional concerns (e.g., health issues) that may be considered for inclusion in the core protocol at all sites or as a specific sub-study focus in the particular site. Community activities will include identification of community representatives and resources and recruitment of community partners to facilitate engagement. Examples include advance contact with community leaders to gather information about the community, town meetings, and listening sessions. Key community members will be recruited and engaged in support of the Study in activities such as acting as a spokesperson for the Study, providing insight into local issues to enhance the relevance of the NCS for their community's health, and serving on community advisory boards. Reliance on secondary data sources like environmental and geographic data can actually enhance these activities. Previous studies have shown the importance of involving community members, either in the actual data collection for the study or as liaisons to special populations such as the medically underserved. These approaches will be utilized at the Study Centers to the extent possible.

Prior to the enrollment period, each Study Center will increase the awareness of the Study among community residents. Building on the community engagement efforts and involvement of community members described above, a variety of strategies will be used to announce the NCS enrollment period. Examples include press releases, appearances on local television and radio shows, and other methods to increase community excitement and interest. Wherever possible, these activities will involve joint participation of study staff and community members. These press and public relations activities will have the technical support of the Coordinating Center and the NCS Program Office, with the approval of the NCS Project Officer.

Throughout the Study, the Study Centers will involve and solicit input from the community. Examples of ongoing activities include establishing a community advisory board, partnering with other organizations to host events or forums, incorporating community leaders into the Study Center structure, and building referral networks between the Study and organizations. Steps for community engagement will vary depending on the characteristics and experiences of the communities and the Centers, and it is expected that the most effective approaches will vary. Once data collection begins, communities will be interested in learning about Study findings. Aggregate findings will be shared with individual participants and communities through newsletters, publications, and other means. The community perspective can inform NCS researchers on ways to be sensitive to unique cultural and political issues and to concerns

within each community when communicating results. Because the NCS is a long-term research effort, attention to sustaining community relationships will be very important.

6.6 Data Collection Schedule

The following section provides an overview of the data collection schedule at each contact until the child reaches age 1. For details about the measurements (e.g. content of questionnaires, targeted analytes in biological or environmental samples, or specifics of physical assessments) and their relation to hypotheses, see Appendices E through I.

The data collection schedule for the NCS includes a variety of data collection modalities at each participant contact. A comprehensive schedule of in-person visits in the home or a clinical setting, contacts by telephone, contacts through self-administered forms, and other contact methods has been carefully constructed to minimize respondent burden while enabling measurement of key exposures and outcomes at critical points from before pregnancy through the postnatal period and beyond. Although a framework of anticipated contacts with the study participants is provided through age 21, details of the data collections are specified only for the visits occurring before pregnancy, during pregnancy, at and around birth, and during the first 24 months of the child's life, with the most detailed information through 12 months. Less detail is presented for the preschool period, and provisional details only are presented for subsequent data collections. As technology advances new tools should become available to measure key constructs, and specifying measurement strategies too far in advance might serve to limit the use of cutting-edge advances. Thus, in developing the Study's protocol, maximum flexibility has, and will continue to be, retained with respect to specifying the timing and location of participant contacts for the later years of the Study.

Table 6-1. Current Schedule and Site of In-Person Contacts with Study Participants

Prior to pregnancy*: home	3 years: clinic
First trimester: home	5 years***: to be decided
Second trimester: clinic**, ultrasound only	7 years: to be decided
Second trimester: clinic, ultrasound only	9 years: to be decided
Third trimester: clinic, full visit and ultrasound	12 years: to be decided
Birth: delivery location	16 years: to be decided
6 months: home	20 years: to be decided
12 months: home	

* For women enrolled in the pre-pregnancy cohort (see Section 6.4.2)

** Only if the woman has not had early clinical ultrasound for gestational age dating

***Timing and location of visits from 5 years onward is provisional

6.6.1 Prior to Pregnancy

As described in Section 6.4.2, women who are determined to be at "high probability of pregnancy" will be invited to enroll in the Study's pre-pregnancy cohort. The first data collection for this group will be in the home prior to pregnancy and will include an interview with the enrolled woman, collection of biological specimens and environmental samples, and a brief physical examination. At this visit, women will also be given dietary questionnaires to complete and return to the Study Center. Multiple pregnancy test kits also will be provided and the women will be instructed to use them around the time of their expected menses to enable identification of pregnancy as early as possible. As soon as a woman learns she is pregnant, she will be asked to obtain a self-collected urine sample for assessment of

transient environmental exposures and to contact her local Study Center. For a woman who is not reporting a positive pregnancy test, a series of telephone contacts will occur beginning one month after the initial home visit to ascertain if she has become pregnant and to update contact and environmental exposure information. If after four months there is no pregnancy, the woman will be moved to a lower probability of pregnancy group (either low probability or moderate probability, as defined in Section 6.4.2).

After the initial screening visit, women who are determined to have a low or moderate probability of pregnancy will be asked to contact the Study if their intent to become pregnant changes or if they become pregnant. Both groups will be contacted by telephone by Study Staff every six months for the group with a moderate probability of pregnancy and yearly for the low probability group. The phone contacts will be used to confirm that there has been no change in residence, that the female is still eligible for the Study, and that there has been no change in their probability of pregnancy. If a woman in either the low or moderate probability group became pregnant during the four-year enrollment period, she would be invited to participate in the Study beginning with the appropriate pregnancy visit. Women at low or moderate risk of pregnancy at the initial screening who later move to the higher probability group (e.g., women using reliable birth control who, on rescreening, are no longer using birth control and are actively trying to become pregnant) will be invited to participate in the preconception cohort.

Summary of preconception visits for women with a high probability of pregnancy

- Initial preconception visit (home)
- One month following initial visit (phone)
- Two months following initial visit (phone)
- Four months following initial visit (phone)

6.6.2 Pregnancy

6.6.2.1 Pregnant Women

Two face-to-face visits, one visit for a fetal ultrasound, one more comprehensive clinical visit (including an ultrasound and other assessments), and several phone contacts are planned during pregnancy. The first visit is an in-person contact that will occur as early as possible during pregnancy and will be conducted in the home to allow collection of exposure data during this critical period of early development. In addition to environmental samples taken from the home, the visit will include collection of interview data, biospecimens, and a brief physical examination. Women will be given instructions for completing several self-administered questionnaires, which they will be asked to complete and to mail back after the visit. They will also be provided with a diary to record targeted exposures (e.g., fever) that might be subject to recall bias if ascertained only at planned contacts. Finally, the women will be provided with a health visit log to document visits to clinical providers as well as targeted data items (e.g., blood pressure) (Tang, Ash, Bates, Overhage, & Sands, 2006). Women will be contacted by telephone at approximately 16-17 weeks of gestation to update pregnancy information and environmental exposures. In the mid to late second trimester (approximately 22-24 weeks), women will be invited to receive an NCS-sponsored fetal ultrasound to assess fetal growth. The second core face-to-face data collection will occur in a clinical setting in the early third trimester (approximately 28-30 weeks). The clinical setting was chosen because it can help facilitate the collection of a second standardized

assessment of fetal growth by ultrasound and the collection of other biological specimens and physical assessments. There will also be a brief interview, and women will be given instructions for obtaining easily collected environmental samples from the home that will be mailed back to the Study Center. A telephone contact will again be made at about 36 weeks gestation to update delivery information (i.e., due date, hospital).

6.6.2.2 Early Dating Ultrasound

The Study recognizes the importance of obtaining an early ultrasound to date the pregnancy, to pinpoint the timing of exposures with respect to gestational age, and to assess targeted outcomes accurately, such as preterm birth or fetal and infant growth. Preliminary data suggest that between 40-70 percent of pregnant women from the initial NCS Vanguard Sites will receive a first trimester ultrasound. Thus, at the first Study visit during pregnancy, women will be asked if they already had an ultrasound or if they are scheduled for an early ultrasound. If the answer to either is yes, they will be asked to provide the name of the provider and the needed permissions (e.g., consent and Health Insurance Portability and Accountability Act [HIPAA]) for the Study to obtain results of that ultrasound from the provider. For the women who did not receive an early ultrasound as part of routine care, an ultrasound will be scheduled through the Study. This process was chosen both to decrease the mother's burden and the Study's cost.

6.6.2.3 Biological Fathers

Biological fathers will be invited to participate in the Study. During pregnancy, the primary data collection from fathers is at the time of the first trimester home visit. Targeted data collections include biological specimens, interview data, and a brief physical examination. If an enrolled woman does not want to reveal the identity of the biological father or does not want the Study to contact the biological father, the Study will not contact him. In these instances, the pregnant woman (and her child) would still be eligible for participation in the Study. The father does not necessarily need to live in the same home as the mother for initial inclusion in the Study, however, biological fathers or biological mothers who have no contact with the child following birth will not be followed.

Summary of pregnancy visits

- Early first trimester: (home: mother and biological father)
- First trimester (clinic: ultrasound for women without an early clinical ultrasound)
- 16-17 weeks (phone contact: mother)
- 22-24 weeks (clinical visit, ultrasound only: mother)
- 28-30 weeks (clinical visit with ultrasound: mother)
- 36 weeks (phone: mother)

6.6.3 Following Pregnancy

A number of data collections central to the Study occur at the birth location around the time of delivery. These include a brief maternal interview; the collection of biological specimens (e.g., cord blood, placental tissue, and meconium); information about the delivery and the hospital course of the infant as ascertained through abstraction of obstetric and neonatal hospital records; and a baseline neonatal physical and neurodevelopmental assessment. These collections should require at least two visits by Study staff to the place of delivery - one around the time of delivery and a second prior to the infant's discharge. Although the goal is to complete a physical and neurodevelopmental assessment of the child before discharge from the hospital, it is recognized that this will not always be feasible. Thus, if time does not permit assessment in the hospital, a home visit will be made at approximately 1 month after birth.

Following birth, alternating phone and in-person contacts are scheduled every 3 months through age 1 (3-month phone, 6-month in-home visit, 9-month phone, and 1 year in-home visit). After age 1, contacts are every 6 months through age 3 (18, 24 and 30 month phone contacts, and 3-year clinic visit). All contacts include interviews with the primary caregiver to assess both exposures and outcomes of interest. At the 6- and 12-month visits, the primary caregiver and the alternate caregiver (as identified by the primary caregiver) will be interviewed. During each in-person visit, the child will be assessed directly for growth and development and child-parent interactions will be observed. At the home visits, both environmental samples and observational data will be collected. Biological samples will be collected from the child primarily at the 12-month and 3-year visits, although urine will be collected more frequently for measurements of transient environmental exposures. The 3-year clinic visit provides the first opportunity for the measurement of physiologic and physical outcomes (e.g., lung function and body composition) that require larger equipment more easily operated and standardized in a clinical setting. The only biological samples obtained from parents following the birth of the child are salivary samples to measure cortisol as a biomarker of stress, because parental stress is anticipated to have a direct effect on parenting behaviors and child outcomes.

A number of self-administered data collection tools, primarily mail-in questionnaires, will be utilized for more in-depth assessment of some topics than is feasible during the home or clinic visits. Finally, comparable to the data collections during pregnancy, a health visit log for the child will be provided for collection of basic information about clinical visits, including date of visit, type of visit (well child vs. acute), diagnosis, immunizations, etc. Strategies and formats to make the health visit log most valuable to (and thus most utilized by) the participant will be explored during the pilot phase of the Study.

Summary of birth/postnatal visits through 24 months

- Visits around the time of delivery at the place of delivery
- Three months (phone)
- Six months (home)
- Nine months (phone)
- Twelve months (home)
- Eighteen months (phone)
- Twenty-four months (phone)

6.7 Overview of Data Collection for Participant Contacts through 24 Months

This section consists of three tables outlining the contacts between the NCS and Study participants from before pregnancy through the 24-month phone contact. The relations between each participant contact, the relevant data collection modalities for that contact, and the broad domains assessed during that contact are illustrated. Table 6-2 shows the pre-pregnancy and pregnancy contacts for the mother, Table 6-3 outlines the maternal and child contacts from birth through 24 months, and Table 6-4 shows partner contacts.

The appendices include more detailed text and tabular descriptions of the NCS data collection activities. Appendix D describes each of the data collection modalities, and Appendices E through I describe specific exposure and outcome measures as well as potential confounders that are being assessed.

Table 6-2. Prepregnancy and Pregnancy: Maternal Contacts

Visit	Questionnaire and Diary	Biologic Samples	Clinical/Developmental Examination	Environmental Samples
Prepregnancy home visit	Maternal interview Demographics Household composition Medication use Health behaviors Housing characteristics Chemical exposures Product use Occupational exposures Diet	Blood Urine Saliva Vaginal swabs Hair	Anthropometrics Blood pressure	Indoor air House dust
Prepregnancy phone follow-up	Maternal phone interview Diet Chemical exposures	------	-------	-----
First trimester home visit	Maternal interview Demographics* Household composition* Medication use* Health behaviors* Housing characteristics* Chemical exposures* Product use* Occupational exposures* Diet* Medical history Stress and social support Depression	Blood Urine Saliva Vaginal swabs Hair	Anthropometrics Blood pressure Fetal ultrasound (from medical report or clinic visit)	Indoor air House dust Drinking water Soil
Second trimester phone follow-up	Maternal phone interview Major life events Mental health update Medical update Chemical exposures update Housing update	-----	-----	-----
Third trimester clinic visit	Maternal interview Updates on: Demographics Household composition Medication use Health behaviors Housing characteristics Chemical exposures Product use Occupational exposures Diet Medical history Stress and social support Prenatal life events Depression	Blood Urine Saliva Vaginal swabs Hair	Anthropometrics Blood pressure Fetal ultrasound	Indoor air House dust (self-collected and mailed in)

* Updates if in prepregnancy cohort

Table 6-3. Birth through 24 months: Maternal (M) and Child (C) Contacts

Visit	Questionnaire and Diary	Biologic Samples	Clinical/Developmental Examination	Environmental Samples
Birth: At delivery, hospital	Maternal interview Health behaviors (M) Diet (M) Chemical exposures (M) Plans for infant feeding, sleeping, etc.	Blood (M) Urine (M) Cord blood Placenta and cord samples Heel stick (C)	Anthropometrics (C) Dysmorphology and neurologic exam (C) Digital photographs of face and anomalies (C) Chart abstraction (M, C)	-----
3-month phone call	Maternal phone interview Child care Medical update (C)	Breast milk (mailed in at 4-6 weeks)	-------	-----
6-month home visit	Maternal interview Stress and social support Family process and parenting practices Health behaviors (M) Depression and cognition (M) Diet (C) Medical update (C) Medication use (C) Media exposure (C) Child care Chemical exposures Temperament (C)	Urine (C) Hair (C) Saliva (M) Breast milk	Anthropometrics (C) Dysmorphology exam and photos (C) Dermatologic exam (C) Social development observation (M, C)	Indoor air House dust Drinking water Soil Visual assessment of house and neighborhood
9-month phone call	Maternal phone interview Child care Medical update (C) Housing update Chemical and occupational exposures (M, C)	-----	-----	-----

Table 6-3. Birth through 24 months: Maternal (M) and Child (C) Contacts (continued)

Visit	Questionnaire and Diary	Biologic Samples	Clinical/Developmental Examination	Environmental Samples
12-month home visit	Maternal interview Household composition update Family process and parenting practices Health behaviors (M) Diet (C) Medical update (C) Medication use (C) Media exposure (C) Child care Housing update Chemical and occupational exposures (M, C) Language acquisition and social interaction (C)	Blood (C) Urine (C) Hair (C) Saliva (C) Breast milk	Anthropometrics Blood pressure Dermatologic exam Cognitive exam Motor and language assessments Social development observation (child and father, if available)	Indoor air House dust Drinking water Soil Visual assessment of house and neighborhood Noise survey
18-month phone call	Maternal interview Child care Medical update (C) Diet (C) Housing update Chemical and occupational exposures (M, C)	-----	-----	-----
24-month phone call	Maternal interview Child care Medical update (C) Housing update Chemical and occupational exposures (M, C) Life events (M)	-----	-----	Indoor air House dust (self-collected and mailed in)

Table 6-4. Paternal or Partner Contacts

Visit	Questionnaire	Biologic Samples	Clinical/Developmental Examination
First trimester home visit	Partner interview Demographics Household composition Tobacco use Medical history Cognition	Blood Urine Hair	Anthropometrics Blood pressure
6-month home visit	Partner interview Family process and parenting practice Tobacco use Mental health	Saliva	-----
12-month home visit	Partner interview Family process and parenting practice Tobacco use Cognition (if not assessed at first trimester visit)	-----	Social development observation with child

Chapter 7

Selection of Outcome and Exposure Measures

7. SELECTION OF OUTCOME AND EXPOSURE MEASURES

To guide the selection and prioritization of exposure and outcome measures, 26 hypotheses were developed based on the input from multiple federal agencies and scientific experts. The criteria for these core hypotheses were that they be scientifically compelling, have important public health implications, be feasible to test, and clearly justify the need for a prospective birth cohort study of 100,000. These hypotheses can be found in Appendix A. Although it is expected that many other scientific questions will be investigated, the core hypotheses have served as guidelines for prioritization of measures.

Due to the breadth of the National Children's Study, each contact between the participant and NCS personnel must capture information pertinent to multiple exposure and outcome domains. Thus, the length of time a measure takes to administer is an important issue with respect to overall participant burden and retention. Also for these reasons, measures that are generally perceived as invasive or uncomfortable are less likely to be included in the full protocol. Each of the procedures, measurements, and assessments associated with the NCS must meet the criteria for "minimal risk" as defined in the Code of Federal Regulations [§45 CFR 46.102(i)]. In addition, the NCS is committed to minimizing even minimal risks.

7.1 Exposures, Outcomes, Mediators, and Confounders

To some degree, the categorization of certain measurements as an outcome, an exposure, a potential mediator, or a confounder is arbitrary because a factor that is an exposure in one hypothesis may be a mediator, confounder, or outcome for another. For example, childhood obesity can be considered an outcome related to fetal growth and maternal glucose tolerance and also a risk factor for subsequent development of diabetes or cardiovascular disease. For consistency, this document categorizes outcomes and exposures as they are presented in the NCS hypotheses while acknowledging the fluidity inherent in many of the areas. For a more in-depth discussion of mediators and confounders see the Statistical Analysis Plan (Chapter 10).

7.2 Overview of Measures

As described previously, the NCS will engage in a continual process of planning the protocol measures to ensure they reflect the best science and technology available. Protocol development will continue as the children age and new scientific data become available. Specific measurements for each new wave of data collection will begin approximately two years before the measures are needed in the field.

Assessment of exposures and outcomes will utilize tools suitable for a large-scale, longitudinal, multi-site, geographically dispersed epidemiologic study. In general, measurements used successfully in other large studies of child health are most likely to be included in the NCS because they have a record of past performance and will facilitate the comparison of NCS results to those from other studies. However, some novel or less frequently used tools may provide important high-quality data and are included where appropriate for the entire cohort, while others may be more suitable for focused adjunct studies.

7.2.1 Organization of Rationale for Measures Chapters and Appendices

The rationale for outcome and exposure measurement strategies is presented in Chapters 8 and 9. The domains of exposures and outcomes described in these chapters extend from birth to age 21. There is more specificity, however, relating to assessments through infancy since protocol development is still ongoing. These chapters are supported by Appendices E through I, which contain detailed information about the domains of measurement at each participant contact through child age 24 months.

Chapter 8 describes the rationale for measurement strategies with regard to each of the seven priority outcome areas: pregnancy; neurodevelopment and behavior; child health and development; asthma; obesity and growth; injury; and reproductive development.

Chapter 9 describes the rationale for measurement strategies with regard to chemical exposures, physical exposures and environment, psychosocial environment, biological exposures, and genetics.

7.2.2 Overview of Measures Related to Specific Hypotheses

As mentioned previously, the NCS core hypotheses served as guidelines for the selection of outcome and exposure measurement domains. Consequently, in conceptualizing the relation between the outcome and the exposure measurement domains, it is helpful to think about their connections within these core hypotheses. Table 7-1 presents the 26 core hypotheses across the top of the table organized by priority outcome area. Domains of exposures and covariates are listed down the side. For each hypothesis, the exposures and covariates central to that hypothesis are indicated in the table.

Table 7-1. Measures by Hypotheses

	Pregnancy Outcomes					Neurodevelopment and Behavior			Child Health and Development				Asthma					Obesity and Growth					Injury			Reproductive Development
	Birth Defects from Impaired Glucose Metabolism	Increased Risk of Preterm Birth from Intrauterine Exposure to Mediators of Inflammation	Increased risk of fetal growth restriction, birth defects, and disabilities in children born through assisted reproductive technologies	Maternal Subclinical Hypothyroidism and adverse pregnancy outcomes	Nonpersistent Pesticides and Poor Neurobehavioral and Cognitive Skills	Prenatal Infection and Neurodevelopmental Disabilities	Gene Environment Interactions and Behavior	Prenatal and Perinatal Infection and Schizophrenia	Family Influences on Child Health and Development	Impact of Neighborhood and Communities on Child Health	Impact of Media Exposure on Child Health and Development	Social Institutions and Child Health and Development	Prenatal Maternal Stress and Genetics in Childhood Asthma	Indoor, Outdoor Air Pollution, Aeroallergens and Asthma Risk	Dietary Antioxidants and Asthma Risk	Social Environmental influences on asthma disparities	Early Exposure to Components and Products of Microgaanisms Decreases the Risk of Asthma	Obesity and Insulin Resistance from Impaired Maternal Glucose Metabolism	Obesity and Insulin Resistance form Intrauterine Growth Restriction	Breastfeeding Associated with Lower Rates of Obesity and Lower Risk of Insulin Resistance	Fiber, Whole Grains, High Glycemic Index and Obesity, Insulin Resistance	Genetics, Environmental Exposures, and Type I Diabetes	Repeated Mild Traumatic Brain Injury and Neurocognitive Development	Behavioral Exposures, Genetics, and Childhood or Adolescent Onset Agression	Antecedents and Resiliency to Traumatic Life Events in Childhood	Hormonally Active Environmental Agents and Reproductive Development
Questionnaire - Mother																										
HH Composition and Demographics		C	C		C		C	C	N	C	C	C	C	C		C	C	C	C	C	C		C		N	C
Parental Stress		C				C	N		N	N			N											N	N	
Maternal Exhaustion *Prop							C						C											C		
Social Support							N		C				C			C								C		
Family Process							N		N	C						N				C			N	N	C	
Health Behaviors	C	C					C				C		C	C				C	C	C	C			C		C
Diet and Toxicants through food					N										N		N	C	C	N	N	N				C
Media Use									C		N	C														
Maternal Mental Health & Cognition					C		N		N		C	C												N	N	
Parenting Style							N		N		C													N	N	
Maternal / Paternal Attachment					N		N		N			N														
Child Care									N	N	N	N														
Neighborhood									C	N		C													N	
Public Policy																									N	
Housing Characteristics				C	C	C	C	C		C				N	C	C	C			C	C		C			C
Occupation / Hobbies				N	N	C	C	C					N	N	C	N	N			C	C	N	C			N
Appliance and Product Use				N	N	C	C	C						N	C	N	N			C	C		C			N
Use of medicines	C					N	C	N							C		C			C	C			C		
Time and Activity (mother / resident father)				N	N	C	C	C			C			N	C	N		C	C	C	C		C			N
Family Environment (observation only)							N		N															N	C	
Brief Medical History	N	N	N		C	N		N	N	N	N	C	N	N	N	N	N	N	N	N	N	N	N	N	N	C
Child Language Development					N		N		N			N														
Child Temperament / Emotional Regulation							N		N															N	C	
Socio-Emotional Functioning					N				N	N	N	N														
Social Competence / Behavior Problems																									C	
Child Autism Screening					N		N		N															N		
Adaptive Behavior							N		C															N		
Questionnaire - Child																										
Neonatal Neuro-behavior	N		N		N	N				N																
General Cognitive Ability					N				N	N	N	N														
General Motor Development					N				N	N	N	N													N	
Cognitive Processes					N	N			N	N	N	N											N	N	N	
Language Development					N				N	N	N	N													N	
Infant Emotion					N		N		N															N	N	
Parent-Child Interaction							N		N															N	N	
Attachment Status					N				N	N	N	N													N	
Sensory Function					N				N	N	N	N														
Questionnaire - Father																										
Demographics									N	C		C													N	
Paternal Mental Health and Cognition					C		N		N		C	C												N	N	
Social Support							N		C				C			C								C		
Experiences with Target Child																										

N = Needed for Analysis C = Confounder/Covariate *Prop = Proposed measure

Table 7-1. Measures by Hypotheses (continued)

	Pregnancy Outcomes				Neurodevelopment and Behavior				Child Health and Development				Asthma					Obesity and Growth					Injury		Reproductive Development
	Birth Defects from Impaired Glucose Metabolism	Increased Risk of Preterm Birth from Intrauterine Exposure to Mediators of Inflammation	Increased risk of fetal growth restriction, birth defects, and disabilities in children born through assisted reproductive technologies	Maternal Subclinical Hypothyroidism and adverse pregnancy outcomes	Nonpersistent Pesticides and Poor Neurobehavioral and Cognitive Skills	Prenatal Infection and Neurodevelopmental Disabilities	Gene Environment Interactions and Behavior	Prenatal and Perinatal Infection and Schizophrenia	Family Influences on Child Health and Development	Impact of Neighborhood and Communities on Child Health	Impact of Media Exposure on Child Health and Development	Social Institutions and Child Health and Development	Prenatal Maternal Stress and Genetics in Childhood Asthma	Indoor, Outdoor Air Pollution, Aeroallergens and Asthma Risk	Dietary Antioxidants and Asthma Risk	Social Environmental Influences on asthma disparities	Early Exposure to Components and Products of Microorganisms Decreases the Risk of Asthma	Obesity and Insulin Resistance from Impaired Maternal Glucose Metabolism	Obesity and Insulin Resistance form Intrauterine Growth Restriction	Breastfeeding Associated with Lower Rates of Obesity and Lower Risk of Insulin Resistance	Fiber, Whole Grains, High Glycemic Index and Obesity, Insulin Resistance	Genetics, Environmental Exposures, and Type I Diabetes	Repeated Mild Traumatic Brain Injury and Neurocognitive Development	Behavioral Exposures, Genetics, and Childhood or Adolescent Onset Agression	Antecedents and Resiliency to Traumatic Life Events in Childhood	Hormonally Active Environmental Agents and Reproductive Development
Questionnaire - Father continued																									
Parenting Style							N		N		C													N	N	
Paternal Attachment					N		N		N			N														
Child Care									N	N	N	N														
Tobacco Product Use, Alcohol Use, Illicit Drug Use and Prescription Drug Abuse (5.10)																										
Use of Medicines and Alternative Medicines																										
Brief Medical History																									N	
Occupation / Hobbies				N	N									N		N	N					N				N
Family Process							N		N	C		C				N				C			N	N		
**Subdomains are shown if they are a confounder/covariate only.																										
Environmental																										
Indoor air																										
PM2.5 - ETS, Pb, Cd, Mn	C	C	C		C	C								N	C	N										N
PM10 - PAHs, pesticides	C	C	C	N	N	C	N,C	C						N		N							C			
VOCs					C	C		C						N									C			
Aldehydes & Ketones							C							N	C	C										
NO2														N	C	N										
Hg				N	C	C	C	C														C	C			
O3														N	C	N										
CO					C									N	C	N										
House dust																										
Allergens, endotoxin														N	C	N										
Mold														N	C	N										
Metals - Pb, Cd, Mn, As					C	C	C																			N
Pesticides: OPs, Carbamates, Pyrethroids				N	N	C	C	C															C			
Pesticides: OCs				N		C	C	C															C			
Drinking water																										
Disinfection Byproducts (DBPs) - HAA9	C	C	C	C				C																		
VOCs	C	C	C		N,C	N,C	N,C	C						N									C			
Metals - Pb, Cd, As			C	C	C	C	C	C															C			N
Nitrate	N,C	N,C	N,C	N,C																						
Perchlorate				N																						N
Pesticides: OPs, Carbamates, Pyrethroids				N	N	C	C	C															C			
Pesticides: Atrazine																										N
Pesticides: OCs				N																						
Soil																										
Metals - Pb, Cd, Mn, As						C	C																			N
Pesticides - OPs, Carbamates, Pyrethroids				N	N	C	C	C															C			
Near CCA treated wood - Cr+6 (as total), As					N	C	C	C															C			
Visual assessment		C		N	N,C	C		C						N,C	C	N,C		C	C				C			N
Noise survey													C													
Indoor/Outdoor measurements																										

N = Needed for Analysis C = Confounder/Covariate *Prop = Proposed measure

Table 7-1. Measures by Hypotheses (continued)

	Pregnancy Outcomes					Neurodevelopment and Behavior			Child Health and Development				Asthma					Obesity and Growth					Injury			Reproductive Development
	Birth Defects from Impaired Glucose Metabolism	Increased Risk of Preterm Birth from Intrauterine Exposure to Mediators of Inflammation	Increased risk of fetal growth restriction, birth defects, and disabilities in children born through assisted reproductive technologies	Maternal Subclinical Hypothyroidism and adverse pregnancy outcomes	Nonpersistent Pesticides and Poor Neurobehavioral and Cognitive Skills	Prenatal Infection and Neurodevelopmental Disabilities	Gene Environment Interactions and Behavior	Prenatal and Perinatal Infection and Schizophrenia	Family Influences on Child Health and Development	Impact of Neighborhood and Communities on Child Health	Impact of Media Exposure on Child Health and Development	Social Institutions and Child Health and Development	Prenatal Maternal Stress and Genetics in Childhood Asthma	Indoor, Outdoor Air Pollution, Aeroallergens and Asthma Risk	Dietary Antioxidants and Asthma Risk	Social Environmental influences on asthma disparities	Early Exposure to Components and Products of Microganisms Decreases the Risk of Asthma	Obesity and Insulin Resistance from Impaired Maternal Glucose Metabolism	Obesity and Insulin Resistance form Intrauterine Growth Restriction	Breastfeeding Associated with Lower Rates of Obesity and Lower Risk of Insulin Resistance	Fiber, Whole Grains, High Glycemic Index and Obesity, Insulin Resistance	Genetics, Environmental Exposures, and Type I Diabetes	Repeated Mild Traumatic Brain Injury and Neurocognitive Development	Behavioral Exposures, Genetics, and Childhood or Adolescent Onset Agression	Antecedents and Resiliency to Traumatic Life Events in Childhood	Hormonally Active Environmental Agents and Reproductive Development
Environmental - Continued																										
Community air																										
PM2.5								C						N	C	N										N
PM10 - PAHs				N	N	C	C	C						C									C			
O_3														N	C	N										
NO_x, SO_2														N	C	N										
Pollen (non-NCS data collection)										C			C	N		N										
Outdoor water samples at homes	C	C	C	N	N	C		C						C			N					C	C			N
Community water systems (non-NCS data collection)																										
Hg				N	C	C	C	C														C	C			
Perchlorate				N																						N
Pesticides				N	N	C	C	C															C			N
Nitrate	C	C	C																							
Physical Exam - Mother																										
Anthropometric									C	C	C							C	C	C	C					C
Ultrasound			N															N	N							
Physical Exam - Father																										
Anthropometric									C	C	C							C	C	C	C					C
Blood Pressure									C	C																
Physical Exam - Child																										
Anthropometric									N	N	N							N	N	N						
Blood Pressure									N	N																
Dysmorphology/Physical Exam	N		N							N																
Physical Activity									C		C							C	C		C					
Biospecimen Collection - Mother																										
Blood																										
Endocrine Panel																										
Cortisol	C						C	N					N										C			
Cortisone	C						C	N					N										C			
Corticotropin releasing hormone (check volume)	C						C	N					N										C			
Cortisol binding globulin	C						C	N					N										C			
CRH binding protein	C						C	N					N										C			
Reproductive																										
Estriol	C						C	N					N										C			
Estradiol	C						C	N					N										C			
Progesterone	C						C	N					N										C			
Infection/Inflammation/Biological																										
CBC (WBC, RBC, Hgb, Hct, MCV,MCH, MCHc, RDW, Plt, MPV, (NE, LY, MO, EO, BA % and #))		N			N	N		N						N			N									
cytokines/interleukins		N				N		N									N									
Ig types and subtypes and org specific		N				N		N																		
Rubella (IgM antibody)		N			C	N		N									N									

N = Needed for Analysis C = Confounder/Covariate *Prop = Proposed measure

Table 7-1. Measures by Hypotheses (continued)

	Pregnancy Outcomes				Neurodevelopment and Behavior				Child Health and Development				Asthma					Obesity and Growth					Injury			Reproductive Development
	Birth Defects from Impaired Glucose Metabolism	Increased Risk of Preterm Birth from Intrauterine Exposure to Mediators of Inflammation	Increased risk of fetal growth restriction, birth defects, and disabilities in children born through assisted reproductive technologies	Maternal Subclinical Hypothyroidism and adverse pregnancy outcomes	Nonpersistent Pesticides and Poor Neurobehavioral and Cognitive Skills	Prenatal Infection and Neurodevelopmental Disabilities	Gene Environment Interactions and Behavior	Prenatal and Perinatal Infection and Schizophrenia	Family Influences on Child Health and Development	Impact of Neighborhood and Communities on Child Health	Impact of Media Exposure on Child Health and Development	Social Institutions and Child Health and Development	Prenatal Maternal Stress and Genetics in Childhood Asthma	Indoor, Outdoor Air Pollution, Aeroallergens and Asthma Risk	Dietary Antioxidants and Asthma Risk	Social Environmental influences on asthma disparities	Early Exposure to Components and Products of Microganisms Decreases the Risk of Asthma	Obesity and Insulin Resistance from Impaired Maternal Glucose Metabolism	Obesity and Insulin Resistance form Intrauterine Growth Restriction	Breastfeeding Associated with Lower Rates of Obesity and Lower Risk of Insulin Resistance	Fiber, Whole Grains, High Glycemic Index and Obesity, Insulin Resistance	Genetics, Environmental Exposures, and Type I Diabetes	Repeated Mild Traumatic Brain Injury and Neurocognitive Development	Behavioral Exposures, Genetics, and Childhood or Adolescent Onset Agression	Antecedents and Resiliency to Traumatic Life Events in Childhood	Hormonally Active Environmental Agents and Reproductive Development
Biospecimen Collection - Mother (continued)																										
Syphilis (Ig)		N			C	N		N									N									
Varicella (Ig)		N			C	N		N									N									
Herpes Simplex 1&2 (Ig)		N			C	N		N									N									
Hepatitis Profile (a and b) *Medical Records* (Ig)		N			C	N		N									N									
Toxoplasmosis (toxoplasma gondii) (Ig)		N			C	N		N									N									
IgE (cat, dog, cockroach, dust mite, fungi, mouse/rat urine)		N				N		N						N			N									
CRP		N			C	N		N									N									
Heat Shock proteins		N			C	N		N									N									
Homocysteine and folate (fasting?) serum or red cells (prenatal vitamin influence)		N			C	N		N							N		N									
Cells T-cell subsets for Th-type *Prop		N			C	N		N									N									
thyroid (TSH and free t4)				N																						
Fasting N3-N6 Fatty Acids *Prop															N											
Antioxidant (vit A/E/Carotenoids)															N											
Vitamin C															N											
%carb deficient transferrin (alcohol)	C	C																						C		C
Glucose Metabolism																										
Fasting C-peptide *Prop	N																	N								
Fasting Glucose	N																	N								
HgbA1C	N																	N								
Insulin like Growth Factor *Prop	N																	N								
Fasting Insulin	N																	N								
Fasting lipids (included in chemical volume)	N																	N								
Genetic Tests																										
DNA, DNA & protein adducts for Exposure Assessment (Chemical Changes)					N																					
Gene Expression (RNA)					N																				N	
Epigenetic changes (genomic DNA)					N																					
Genetic Variation: Paraoxonase Gene, glucokinase, vntr insulin, etc. (DNA)	N				N	C		C										N	N	C	C	C		N		
Cryopreserved PBMCs	N				N	C		C										N	N	C	C	C		N		
Cell lines *Prop	N				N	C		C										N	N	C	C	C		N		
mitochondrial DNA *Prop	N				N	C		C										N	N	C	C	C		N		
Chemical Exposures																										
lipids, PCBs, organochlorine pesiticides, PBDE, Perfluorinated cmpds(PFOA,PFOS) (4 mL Serum)				N	C																					N
Lead, Mercury, Cadmium (3 mL bld)				N	C																			C		N
Combination of dioxins/furans and all other chemicals (excluding metals)				N	C																					N
Stored Samples																										

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Table 7-1. Measures by Hypotheses (continued)

	Pregnancy Outcomes				Neurodevelopment and Behavior				Child Health and Development				Asthma					Obesity and Growth					Injury			Reproductive Development
	Birth Defects from Impaired Glucose Metabolism	Increased Risk of Preterm Birth from Intrauterine Exposure to Mediators of Inflammation	Increased risk of fetal growth restriction, birth defects, and disabilities in children born through assisted reproductive technologies	Maternal Subclinical Hypothyroidism and adverse pregnancy outcomes	Nonpersistent Pesticides and Poor Neurobehavioral and Cognitive Skills	Prenatal Infection and Neurodevelopmental Disabilities	Gene Environment Interactions and Behavior	Prenatal and Perinatal Infection and Schizophrenia	Family Influences on Child Health and Development	Impact of Neighborhood and Communities on Child Health	Impact of Media Exposure on Child Health and Development	Social Institutions and Child Health and Development	Prenatal Maternal Stress and Genetics in Childhood Asthma	Indoor, Outdoor Air Pollution, Aeroallergens and Asthma Risk	Dietary Antioxidants and Asthma Risk	Social Environmental Influences on asthma disparities	Early Exposure to Components and Products of Microganisms Decreases the Risk of Asthma	Obesity and Insulin Resistance from Impaired Maternal Glucose Metabolism	Obesity and Insulin Resistance fom Intrauterine Growth Restriction	Breastfeeding Associated with Lower Rates of Obesity and Lower Risk of Insulin Resistance	Fiber, Whole Grains, High Glycemic Index and Obesity, Insulin Resistance	Genetics, Environmental Exposures, and Type I Diabetes	Repeated Mild Traumatic Brain Injury and Neurocognitive Development	Behavioral Exposures, Genetics, and Childhood or Adolescent Onset Agression	Antecedents and Resiliency to Traumatic Life Events in Childhood	Hormonally Active Environmental Agents and Reproductive Development
Biospecimen Collection - Mother (continued)																										
Serum	N	N		N	N	N		N					N	N	N		N	N								N
Plasma	N	N		N	N	N		N					N	N	N		N	N								N
RBCs (folate and fatty acids)	N	N		N	N	N		N					N	N	N		N	N								N
Lavender Top	N	N		N	N	N		N					N	N	N		N	N								N
Buccal Cells *Prop																										
Nails																										
Organic Hg (ethyl, methyl)				N	C																					N
Hg inorganic				N	C																					N
Hair																										
Cd				N	C																					N
cotinine	C	C		N	N		C						C	N	C									C		C
Hg inorganic				N	C																					N
Organic Hg (ethyl, methyl)				N	C																					N
nicotine	C	C		N	N		C						C	N	C									C	C	C
Saliva																										
Cortisol	C						C	N					N											C		
storage	C						C	N					N											C		
Breast Milk																										
Antioxidants : Vit C/E/ Beta Carotene															N					N						
Component: lipid, proteins, carbohydrates, enzymes, immunoglobulins, minerals, vitamins, cytokines, hormones															N					N						
Chemical Exposures																										
Dioxins/furans; Organochlorine Pesticides; PCBs					C																	N				N
Pesticides					N																	N				N
PBDE (frozen) flame retardant					C																	N				N
Perchlorate					C																	N				N
Metals: Manganese and others					C																	N				N
phytoestrogens					N																	N				N
MTBE methyl tertiary butyl ether (fuel additive)					N																	N				N
Bisphenol A					N																	N				N
Urine																										
Illicit Drug Panel	C	C																						C		C
alcohol marker *Prop	C	C																						C		C
Antioxidants															N											
Catecholamine	C						C						N											C		
Cortisol	C						C	N					N											C		
Asymptomatic bacteriuria		N				N		N									N									
Fertility Monitor																										
Pregnancy Test Kit																										
PCR for identification of specific organisms *Prop		N				N		N									N									
Chemical Exposures																										

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Table 7-1. Measures by Hypotheses (continued)

	Pregnancy Outcomes					Neurodevelopment and Behavior				Child Health and Development				Asthma					Obesity and Growth					Injury			Reproductive Development
	Birth Defects from Impaired Glucose Metabolism	Increased Risk of Preterm Birth from Intrauterine Exposure to Mediators of Inflammation	Increased risk of fetal growth restriction, birth defects, and disabilities in children born through assisted reproductive technologies	Maternal Subclinical Hypothyroidism and adverse pregnancy outcomes	Nonpersistent Pesticides and Poor Neurobehavioral and Cognitive Skills	Prenatal Infection and Neurodevelopmental Disabilities	Gene Environment Interactions and Behavior	Prenatal and Perinatal Infection and Schizophrenia	Family Influences on Child Health and Development	Impact of Neighborhood and Communities on Child Health	Impact of Media Exposure on Child Health and Development	Social Institutions and Child Health and Development	Prenatal Maternal Stress and Genetics in Childhood Asthma	Indoor, Outdoor Air Pollution, Aeroallergens and Asthma Risk	Dietary Antioxidants and Asthma Risk	Social Environmental Influences on asthma disparities	Early Exposure to Components and Products of Microorganisms Decreases the Risk of Asthma	Obesity and Insulin Resistance from Impaired Maternal Glucose Metabolism	Obesity and Insulin Resistance form Intrauterine Growth Restriction	Breastfeeding Associated with Lower Rates of Obesity and Lower Risk of Insulin Resistance	Fiber, Whole Grains, High Glycemic Index and Obesity, Insulin Resistance	Genetics, Environmental Exposures, and Type I Diabetes	Repeated Mild Traumatic Brain Injury and Neurocognitive Development	Behavioral Exposures, Genetics, and Childhood or Adolescent Onset Agression	Antecedents and Resiliency to Traumatic Life Events in Childhood	Hormonally Active Environmental Agents and Reproductive Development
Biospecimen Collection - Mother continued																											
PFBS, Creatinine, alkyl phenols (Bisphenol A, nonylphenol), Hg(inorganic), As(speciated), perchlorate, halogenated phenols(PCP), phthalates, atrazine, OPs, carbamates, pyrethroids, EBDC/ETU, Cadmium				N	N																					N
PAH (may be storage issues); requires separate analysis (3-4 ml)				N	N									N												N
ICP/MS urine screen *Prop				N	C																					N
cotinine	C	C		N	N		C						C	N	C									C		C
Phytoestrogens				N	N										N											N
Storage	C	N		N	N	N	C	N					N	N	N		N							C		N
Vaginal Swabs																										
Chlamydia		N				N		N									N									
Bacterial Vaginosis		N				N		N									N									
Cultures antibodies		N				N		N									N									
Cultures cytokines		N				N		N									N									
Cultures metalloproteinase		N				N		N									N									
Group B Strep		N				N		N									N									
Gonorrhea		N				N		N									N									
Placenta																										
Cultures antibodies and cytokines		N				N		N									N									
Pathology		N				N		N																		
Chemical Contaminants				N	N									N												N
Umbilical Cord																										
Cultures antibodies and cytokines		N				N		N									N									
Pathology		N				N		N																		
Chemical Contaminants				N	N									N												N
Biospecimen Collection - Child																										
Blood (NOTE: Because of the limited blood draw volume for the child, samples will be stored for future analysis. Final protocol for analytes will be decided in the future.)																										
Infection/Inflammation/Biological																										
CBC (WBC, RBC, Hgb, Hct, MCV,MCH, MCHc, RDW, Plt, MPV, (NE, LY, MO, EO, BA % and #))		N			N	N		N		N			N	N		N	N					N				N
Chemical Exposures																										
lipids, PCBs, organochlorine pesiticides, PBDE, Perfluorinated cmpds(PFOA,PFOS) (4 mL Serum)				N	C																	N				N
Stored Samples																										
Serum	N	N		N	N	N	N	N	N	N			N	N	N	N	N	N	N	N	N	N		N		N
Lavender Top	N	N		N	N	N	N	N	N	N			N	N	N	N	N	N	N	N	N	N		N		N
Guthrie Card at birth	N	N		N	N	N	N	N	N	N			N	N	N	N	N	N	N	N	N	N		N		N
Buccal Cells *Prop																										
Hair																										
Cd				N	C																	N				N
cotinine	C	C		N	N		C						C	N	C							N		C		C

N = Needed for Analysis C = Confounder/Covariate *Prop = Proposed measure

Table 7-1. Measures by Hypotheses (continued)

	Pregnancy Outcomes					Neurodevelopment and Behavior				Child Health and Development				Asthma					Obesity and Growth					Injury			Reproductive Development
	Birth Defects from Impaired Glucose Metabolism	Increased Risk of Preterm Birth from Intrauterine Exposure to Mediators of Inflammation	Increased risk of fetal growth restriction, birth defects, and disabilities in children born through assisted reproductive technologies	Maternal Subclinical Hypothyroidism and adverse pregnancy outcomes	Nonpersistent Pesticides and Poor Neurobehavioral and Cognitive Skills	Prenatal Infection and Neurodevelopmental Disabilities	Gene Environment Interactions and Behavior	Prenatal and Perinatal Infection and Schizophrenia	Family Influences on Child Health and Development	Impact of Neighborhood and Communities on Child Health	Impact of Media Exposure on Child Health and Development	Social Institutions and Child Health and Development	Prenatal Maternal Stress and Genetics in Childhood Asthma	Indoor, Outdoor Air Pollution, Aeroallergens and Asthma Risk	Dietary Antioxidants and Asthma Risk	Social Environmental influences on asthma disparities	Early Exposure to Components and Products of Microorganisms Decreases the Risk of Asthma	Obesity and Insulin Resistance from Impaired Maternal Glucose Metabolism	Obesity and Insulin Resistance form Intrauterine Growth Restriction	Breastfeeding Associated with Lower Rates of Obesity and Lower Risk of Insulin Resistance	Fiber, Whole Grains, High Glycemic Index and Obesity, Insulin Resistance	Genetics, Environmental Exposures, and Type I Diabetes	Repeated Mild Traumatic Brain Injury and Neurocognitive Development	Behavioral Exposures, Genetics, and Childhood or Adolescent Onset Agression	Antecedents and Resiliency to Traumatic Life Events in Childhood	Hormonally Active Environmental Agents and Reproductive Development
Biospecimen Collection - Child continued																											
Hg inorganic				N	C																	N				N
Organic Hg (ethyl, methyl)				N	C																	N				N
nicotine	C	C		N	N		C						C	N	C							N		C	C	C
Saliva																										
Cortisol	C						N						N					C	C	C	C	N		N		C
storage	C						N						N					C	C	C	C	N		N		C
Urine																										
Chemical Exposures																										
PFBS, Creatinine, alkyl phenols (Bisphenol A, nonylphenol), Hg(inorganic), As(speciated), perchlorate, halogenated phenols(PCP), phthalates, atrazine, OPs, carbamates, pyrethroids, EBDC/ETU, Cadmium				N	N									N								N				N
PAH (may be storage issues); requires separate analysis (3-4 ml)				N	N									N								N				N
ICP/MS urine screen *Prop				N	C									N								N				N
cotinine	C	C		N	N		C						C	N	C							N		C		C
Phytoestrogens				N	N										N					C	C	N				N
Storage	C	C		N	N	N	C						C	N	N	N	N	N	N	C	C	N		C		N
Meconium																										
Cotinine	C	C		N	N		C						C	N	C							N		C		C
Organophosphate Metabolites				N	N																	N				N
Cord Blood																										
Cortisol	C						N						N					C	C	C	C	N		N		C
Cortisone	C						N						N					C	C	C	C	N		N		C
Corticotropin releasing hormone	C						N						N					C	C	C	C	N		N		C
Cortisol binding globulin	C						N						N					C	C	C	C	N		N		C
CRH binding protein	C						N						N					C	C	C	C	N		N		C
Reproductive																										
Estriol	C						N						N					C	C	C	C	N		N		C
Estradiol	C						N						N					C	C	C	C	N		N		C
Progesterone	C						N						N					C	C	C	C	N		N		C
Infection/Inflammation/Biological																										
CBC (WBC, RBC, Hgb, Hct, MCV,MCH, MCHc, RDW, Plt, MPV, (NE, LY, MO, EO, BA % and #))		N		N	N	N		N		N			N	N		N	N					N				N
cytokines/interleukins		N				N		N		N			N	N		N	N					N				
IgE (cat, dog, cockroach, dust mite, fungi, mouse/rat urine)		N				N		N	N	N			N	N	N	N	N									
Ig types and subtypes		N				N		N	N	N			N	N	N	N	N									
N3-N6 Fatty Acids								C							N			C	C	C	C					
Antioxidant (vit A/E/Carotenoids)								C							N			C	C	C	C					
Vitamin C								C							N			C	C	C	C					
Lymphocyte Subsets Th status (processing difficult)																										
Glucose Metabolism																										
Glucose	N								N	N								N	N	N	N	N				
HgbA1C	N								N	N								N	N	N	N	N				
Insulin like Growth Factor	N								N	N								N	N	N	N	N				

N = Needed for Analysis C = Confounder/Covariate *Prop = Proposed measure

Table 7-1. Measures by Hypotheses (continued)

	Pregnancy Outcomes				Neurodevelopment and Behavior				Child Health and Development				Asthma					Obesity and Growth					Injury		Reproductive Development
	Birth Defects from Impaired Glucose Metabolism	Increased Risk of Preterm Birth from Intrauterine Exposure to Mediators of Inflammation	Increased risk of fetal growth restriction, birth defects, and disabilities in children born through assisted reproductive technologies	Maternal Subclinical Hypothyroidism and adverse pregnancy outcomes	Nonpersistent Pesticides and Poor Neurobehavioral and Cognitive Skills	Prenatal Infection and Neurodevelopmental Disabilities	Gene Environment Interactions and Behavior	Prenatal and Perinatal Infection and Schizophrenia	Family Influences on Child Health and Development	Impact of Neighborhood and Communities on Child Health	Impact of Media Exposure on Child Health and Development	Social Institutions and Child Health and Development	Prenatal Maternal Stress and Genetics in Childhood Asthma	Indoor, Outdoor Air Pollution, Aeroallergens and Asthma Risk	Dietary Antioxidants and Asthma Risk	Social Environmental Influences on asthma disparities	Early Exposure to Components and Products of Microorganisms Decreases the Risk of Asthma	Obesity and Insulin Resistance from Impaired Maternal Glucose Metabolism	Obesity and Insulin Resistance form Intrauterine Growth Restriction	Breastfeeding Associated with Lower Rates of Obesity and Lower Risk of Insulin Resistance	Fiber, Whole Grains, High Glycemic Index and Obesity, Insulin Resistance	Genetics, Environmental Exposures, and Type I Diabetes	Repeated Mild Traumatic Brain Injury and Neurocognitive Development	Behavioral Exposures, Genetics, and Childhood or Adolescent Onset Agression	Antecedents and Resiliency to Traumatic Life Events in Childhood	Hormonally Active Environmental Agents and Reproductive Development
Biospecimen Collection - Child continued																									
Insulin	N								N	N								N	N	N	N	N			
lipids (included in chemical volume)	N								N	N								N	N	N	N	N			
Genetic Tests																									
DNA, DNA & protein adducts for Exposure Assessment (Chemical Changes)					N																	N			
Gene Expression (RNA)					N		N													C	C	N		N	N	
Epigenetic changes (genomic DNA)					N		N													C	C	N		N		
Genetic Variation: Paraoxonase Gene, glucokinase, vntr insulin, etc. (DNA)	N				N	C	N	C										N	N	C	C	N		N		
mitochondrial DNA *Prop	N				N	C	N	C										N	N	C	C	N		N		
Chemical Exposures																									
lipids, PCBs, organochlorine pesiticides, PBDE, Perfluorinated cmpds (4 mL Serum)				N	C																	N			N
Lead, Mercury, Cadmium (3 mL bld)				N	C																	N		C	N
Stored Samples																									
Serum	N	N		N	N	N	N	N	N	N			N	N	N	N	N	N	N	N	N	N		N	N
Plasma	N	N		N	N	N	N	N	N	N			N	N	N	N	N	N	N	N	N	N		N	N
RBCs	N	N		N	N	N	N	N	N	N			N	N	N	N	N	N	N	N	N	N		N	N
Guthrie Card	N	N		N	N	N	N	N	N	N			N	N	N	N	N	N	N	N	N	N		N	N
Lavender Top	N	N		N	N	N	N	N	N	N			N	N	N	N	N	N	N	N	N	N		N	N
Whole blood storage PCR	N	N		N	N	N	N	N	N	N			N	N	N	N	N	N	N	N	N	N		N	N
Biospecimen Collection - Father																									
Blood																									
Genetic Tests																									
DNA, DNA & protein adducts for Exposure Assessment (Chemical Changes)																									
Gene Expression (RNA)																								N	
Epigenetic changes (genomic DNA)																									
Genetic Variation: Paraoxonase Gene, glucokinase, vntr insulin, etc. (DNA)	N				N	C		C										N	N	C	C	C		N	
Cryopreserved PBMCs	N				N	C		C										N	N	C	C	C		N	
Cell lines *Prop	N				N	C		C										N	N	C	C	C		N	
mitochondrial DNA *Prop	N				N	C		C										N	N	C	C	C		N	
Chemical Exposures																									
lipids, PCBs, organochlorine pesiticides, PBDE, Perfluorinated cmpds(PFOA,PFOS) (4 mL Serum)					C																				
Lead, Mercury, Cadmium (3 mL bld)					C																				
Combination of dioxins/furans and all other chemicals (excluding metals)					C																				
Stored Samples																									
Serum					N	N		N																N	
Plasma					N	N		N																N	

N = Needed for Analysis C = Confounder/Covariate *Prop = Proposed measure

Table 7-1. Measures by Hypotheses (continued)

	Pregnancy Outcomes				Neurodevelopment and Behavior				Child Health and Development				Asthma					Obesity and Growth					Injury		Reproductive Development
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Biospecimen Collection - Father continued																									
RBCs (folate and fatty acids)					N	N		N																N		
Lavender Top					N	N		N																N		
Buccal Cells *Prop																										
Nails																										
Organic Hg (ethyl, methyl)					C																					
Hg inorganic					C																					
Hair																										
Cd					C																					
cotinine					N																					
Hg inorganic					C																					
Organic Hg (ethyl, methyl)					C																					
nicotine					N																				C	
Saliva																										
Cortisol	C						C						N											C		
storage	C						C						N											C		
Urine																										
Chemical Exposures																										
PFBS, Creatinine, alkyl phenols (Bisphenol A, nonylphenol), Hg(inorganic), As(speciated), perchlorate, halogenated phenols(PCP), phthalates, atrazine, OPs, carbamates, pyrethroids, EBDC/ETU, Cadmium					N																					
PAH (may be storage issues); requires separate analysis (3-4 ml)					N																					
ICP/MS urine screen *Prop					C																					
cotinine					N		C																			
Phytoestrogens					N																					
Storage					N		N																			
Semen																										
Quality (home collection)																										

N = Needed for Analysis C = Confounder/Covariate *Prop = Proposed measure

Chapter 8

Rationale for Outcome Measures

8. RATIONALE FOR OUTCOME MEASURES

The rationale for outcome measures is divided into subsections, one for each of the seven National Children's Study priority outcome areas. The measures outlined in these subsections will allow the NCS to address important hypotheses relevant to these outcome areas. The broad array of data to be collected by the NCS, however, will also enable the examination of countless additional health and developmental outcomes. Some of the outcomes comprise more or less definitive diagnoses, such as preterm birth or congenital anomalies. Others entail the initial assessments of quantitative trajectories that will be measured throughout the Study, such as cognitive development, behavior, or body composition.

8.1 Pregnancy Outcomes

8.1.1 Definition

Proximal outcomes of pregnancy will be the first measures of child health captured by the NCS. Preterm birth and structural congenital anomalies are of specific interest because of the immediate morbidity and mortality associated with those conditions and the potential persistence of associated morbidity and disability throughout life. In addition, growing evidence suggests that deviation from normal fetal growth trajectories, even if not associated with perinatal complications, may be related to cardiovascular or other chronic conditions later in life (Barker, 1994). Preterm birth is generally defined as birth prior to 37 completed weeks of gestation (calculated as the time from the start of the last menstrual period to the time of birth). A structural birth defect is generally defined as malformation of an organ or structure that is present at birth and adversely affects health and development. In addition to documenting major structural anomalies (e.g., neural tube defects, facial clefts, cardiac defects), the NCS also will attempt to measure subtle variations in morphogenesis that may be related to periconceptional chemical or metabolic exposures and later neurodevelopment.

8.1.2 Assessment of Gestational Age and Fetal Growth

While the majority of preterm births occur close to term and are at relatively low risk for severe morbidity, the relatively small proportion of births at the lower end of the viable gestational age range (down to approximately 24 weeks) are at greatly elevated risk for mortality and long-term morbidity. In addition, the quality of fetal growth assessment, either by growth parameters obtained in utero via ultrasound or by using birth weight and anthropometric measures obtained at birth, is dependent on accurate knowledge of gestational age. Thus accurate ascertainment of gestational age is important not only to identify degree of preterm birth, but also to collect accurate fetal growth measures that may be independent variables of interest for many child outcomes throughout the course of the NCS.

In a clinical setting, a fetus's gestational age is based on the date of the last menstrual period. An increasing proportion of women receive a first trimester ultrasound (generally between 8-12 weeks gestation) that also is used to estimate gestational age. These estimates are commonly based on crown-rump length (Hadlock, Shah, Kanon, & Lindsay, 1992), though algorithms using other measures, such as biparietal diameter, are also used.

Measures of gestational age

In the NCS, data for estimation of gestational age will come from a variety of sources. Among all women, questionnaire data will ascertain date of last menstrual period and whether a first trimester ultrasound was or will be obtained. If so, a report of that scan will be collected and used as a basis for gestational age. If a woman does not receive an early clinical scan, then she will be scheduled for one under the auspices of the NCS. Among women enrolled prior to pregnancy, the use of frequent pregnancy tests may provide an additional, potentially accurate, estimate of date of conception. For all women, date of birth should be directly available since collection of maternal and infant samples at birth is a focus of the NCS. In situations where the birth was missed by the NCS, retrospective interview data and review of the labor and delivery and neonatal charts will be used to ascertain birth date and other perinatal information.

Reconciliation of multiple, sometimes discordant, measures of duration of pregnancy is often neither simple nor straightforward. The various measures are based on three conceptually different constructs (Alexander & Allen, 1996): time (days from menstruation or ovulation to birth), size (sonography), or maturity (newborn examinations such as those of Ballard et al., 1991). Menstrual dating is the traditional gold standard, and all other measures were originally validated among women with well-characterized menstrual dates. Especially on the aggregate level, however, uncritical acceptance of menstrual dates leads to gestational age estimates that may be implausible, manifested particularly as a bimodal distribution of birth weight at early gestational ages and a biologically implausible number of pregnancies continuing considerably beyond the expected 280 days (David, 1980). When sonography was compared directly to menstrual dating, it was found that a menstrual age of less than 37 completed weeks was in agreement with sonography in only 78 percent of cases, and a menstrual age of greater than 42 weeks agreed with sonography in only 11 percent of cases.

Uncritical acceptance of sonography, however, could gloss over subtle differences in growth that may be of etiologic interest. The fundamental assumption underlying sonographic estimation of gestational age is that inter-individual differences in growth are nonexistent early in pregnancy. Recent research has suggested that this assumption is not tenable. For example, compared to menstrual dating, gestational age estimated from the fetal biparietal diameter consistently underestimated the gestational duration of girls compared to boys, and of fetuses of mothers who smoked compared to nonsmokers (Henrihsen, Wilcox, Hedegaard, & Jorgen Secher, 1995; Morin et al., 2005). This suggests that even in the first half of pregnancy, known influences on fetal growth are operative and can impact measurement of gestational age. This phenomenon has recently been reported in first-trimester sonography among pregnancies with a known date of conception (due to in-vitro fertilization). In that study (Bukowski et al., 2007) a fetus whose sonographic estimate of gestation was even one day greater than the known time of conception was less likely to be undergrown, or even preterm, at birth. This suggests that growth differences are present even in the first trimester, and may be of etiologic significance.

For these reasons, the NCS will not have a single "study gestational age." Data will be collected on menstrual history, sonography, and other clinical measures of duration of pregnancy, and individual researchers will be free to explore these intriguing differences further.

Measures of fetal growth

Assessments of fetal growth are based on relative size for a given gestational age. In the NCS, linear measures of growth including biparietal diameter, abdominal circumference, and femur length will be obtained via standardized ultrasounds at approximately 22-24 weeks and 28-30 weeks of gestation. These repeated measures, as well as others obtained from the newborn infant, will enable true

growth rates to be calculated and may enable the NCS to distinguish slow growth in the first half of pregnancy from slow growth occurring later. In addition, though not a routine clinical measure, mid-thigh lean and fat mass circumferences will be obtained. Although it would be ideal to acquire additional growth measures, cost and participant burden constraints will not allow additional study-related visits for standardized ultrasounds. However, if additional clinical scans are performed, relevant growth data from those scans will be collected. At birth, birth weight, length, head and abdominal circumferences, and triceps and subscapular skin folds will be measured on each infant. Birth weight may be compared to external size-for-gestational age standards, such as those of Alexander, Himes, Kaufman, Mor, and Kogan (1996), Zhang and Bowes (1995) or Kramer et al. (2001). Long-term impacts of size at birth may not, however, be limited merely to the smallest percentiles of size-for-dates, but rather may operate continuously across of broad spectrum of relative size (Innes et al., 2003), and therefore the complete continuum of size will be evaluated on the NCS.

8.1.3 Assessment of Congenital Anomalies

Clinical management of pregnancy offers the opportunity for congenital anomaly assessment of several types and at various stages, depending on maternal and familial risk factors. Women more than 35 years old or with a relevant genetic history will generally be offered chorionic villus sampling at 10-12 weeks gestation or, more commonly, amniocentesis around 16 weeks. Early ultrasounds primarily used for confirming gestational age can also be used to ascertain nuchal fold thickness, if performed between 10 and 14 weeks gestation; increased nuchal translucency is associated with increased risk of Down Syndrome, other chromosomal abnormalities, and some cardiac defects. Serum triple or quad screen is generally performed between 16-18 weeks of pregnancy to assess risk for neural tube defects or Down Syndrome. A fetus may also receive one or more anatomic surveys by ultrasound, depending on maternal risk factors and perceived fetal growth. Though commonly performed around the 20th week of pregnancy, anatomic scans are also obtained both earlier and later to assess for structural defects.

Case definition and ascertainment

The NCS will rely primarily on recording clinical diagnosis of major structural anomalies, rather than performing specific diagnostic tests as part of the Study protocol. NCS involvement in diagnosis of anomalies may be confusing to a pregnant woman, who is receiving principal medical care and advice from her clinician. In addition, the NCS is not in a position to provide the necessary counseling and follow-up if tests show positive or even equivocal results. Although the NCS ultrasounds are focused on measuring fetal growth, procedures for referral and follow-up, if abnormalities are noted, will be included in the manual of operations.

The prevalence of congenital anomalies depends heavily on the period of ascertainment, prenatal diagnosis, elective terminations, and the data sources reviewed. For example, the percentage of structural defects diagnosed by ultrasonography increases by the trimester of pregnancy (Withlow, Chatzipapas, Lazanakis, Kadir, & Economides, 1999). In addition, a pregnancy may be terminated before the time of viability because of a serious or lethal defect, and among spontaneous pregnancy losses and fetal deaths the prevalence of structural malformations declines as pregnancy progresses (Dimmick & Kalousek, 1992). Thus, although the NCS will rely primarily on existing clinical diagnosis, records from every spontaneous abortion, stillbirth, and elective termination will be obtained whenever possible to determine the presence of a structural defect in the fetus. Should a Study- or clinically-obtained sonogram detect an anomaly and the pregnancy not be terminated, birth records will be abstracted to determine the ultimate diagnosis; terminations because of defects detected sonographically will be confirmed by review of medical and/or pathology records whenever possible. Since the vast majority of pregnancies in the

NCS will have been ascertained in the first trimester, such complete surveillance will be technically feasible.

Review of the maternal and infant charts at birth, as well as maternal questionnaires during pregnancy, will be used to ascertain prenatal diagnosis of congenital anomalies. After birth, a standardized observational infant exam will record any major anomalies. Selected morphologic measurements, such as intercanthal distances and anogenital distance, also will be made at that exam. A standardized digital facial photograph will be taken at birth and stored for later analysis. Digital photographs of external anomalies will also be taken. Questionnaire data regarding medical diagnoses and information recorded in the child's health care visit log after birth will be used to identify birth defects not recorded at the birth visit. The accuracy of questionnaire reports of birth defects, however, has been called into question (Rasmussen, Mulinare, Khoury, & Maloney, 1990; Romitti, Burns, & Murray, 1997). Therefore, potentially major birth defects identified through questionnaire will be further investigated. To the extent possible, review of extant medical data, including physical examinations, operative notes, autopsy records, cytogenetic and metabolic studies, and/or imaging studies, will be used to identify and characterize major congenital anomalies accurately. Furthermore, a considerable fraction of all defects are not apparent at birth but only become known over time. This is particularly the case for defects of the internal organs, such as the heart and kidneys. Parents/guardians of all Study children will be interviewed on multiple occasions during the first 5 years of life and on each occasion will be asked about any diagnosis of birth defects as well as any operations or significant medical procedures. If these screenings suggest the presence of any defect, relevant records will be obtained for confirmation whenever possible, following procedures similar to those used by active birth defects surveillance programs (Correa et al., 2007).

Case classification

Researchers studying birth defects recognize that application of current knowledge of embryology and pathophysiology is essential when classifying these conditions. It is inappropriate simply to classify defects as any versus none, or even by organ system (e.g., all heart defects, all limb defects, etc.). Such classification ignores the etiologic heterogeneity present in these defects. Furthermore, researchers distinguish between infants with an isolated defect, a known malformation syndrome, a sequence (i.e., multiple defects that are the result of a single primary defect), or other complex sets of defects. Etiology can reflect malformations (i.e., a localized error in morphogenesis), deformations (alteration of an otherwise normally developing structure, usually by mechanical forces), disruptions (destruction of a normally formed structure, usually by vascular, mechanical, or infectious insults) or dysplasias (lack of normal organization of cells into tissues).

Distinguishing between these subtleties requires a thorough review of all available information on each child in order to classify defects in an etiologically homogeneous manner. As noted above, the information collected by the NCS will enable detailed, specific review of the information on each case by a group of experts in the relevant field, for example, a pediatric cardiologist, a clinical geneticist, etc. While such review may not necessarily be done in "real time," appropriate data will be collected to enable future research to make classifications based on the most current science.

8.1.4 Assessment of Other Pregnancy Outcomes

Information on the occurrence of miscarriage or stillbirth will be ascertained via maternal questionnaire. At each regular contact with an enrolled pregnant woman, the woman will be asked how the pregnancy is progressing. If the woman indicates she is no longer pregnant, she will be asked for

further information regarding when the loss occurred. When possible, additional diagnostic information, including evidence of chromosomal malformations or birth defects, will be obtained from medical or post-mortem records. A standardized procedure for examination of stillbirths similar to that used by the NICHD Stillbirth Collaborative Research Network may be possible in some Study sites, but will be difficult to implement universally due to the number and diversity of medical care systems involved in the NCS.

8.1.5 Assessment of Related Factors

Experiences during pregnancy, particularly maternal medical status, have been linked to adverse pregnancy outcomes. Suboptimal thyroid function in pregnancy is associated with risk for preterm birth. Impaired glucose metabolism during pregnancy is associated with a variety to congenital anomalies, including malformations of the heart, central nervous system, and musculoskeletal system. Maternal infection, and thus fetal exposure to mediators of inflammation due to maternal infection, has also been implicated in preterm birth. The NCS will assess maternal medical status and other maternal exposures repeatedly during pregnancy.

8.2 Neurodevelopment and Behavior

8.2.1 Definition

Children's achievement of age-normative levels of developmental functioning, and non-normative deviations from those developmental milestones, will be of great concern on the NCS. The domain of neurodevelopment and behavior, which includes neurocognitive and motor functioning, attentional abilities, social functioning, and behavior regulation, will be assessed at multiple time points. Identification of both symptoms of disorder, and of specific developmental, behavioral, or mental health disorders—conditions such as autism spectrum disorders, attention deficit-hyperactivity disorder, anxiety disorders, depression, or schizophrenia—will be identified using a number of modalities, as discussed below. Details about neurodevelopment and behavior measures appear in Appendix F.1.

8.2.2 Assessment of Developmental and Mental Health Problems and Disorders

Diagnoses of specific behavioral or mental health conditions in clinical research are generally based on a patient's history, patient-provider interaction, and the use of condition-specific diagnostic tools, such as the Autism Diagnostic Observation Schedule and Autism Diagnostic Interview-Revised for autism. Actual diagnosis of specified conditions is currently defined by the International Classification of Diseases-Clinical Modification of the World Health Organization; the Diagnostic and Statistical Manual of Mental Disorders-IV of the American Psychiatric Association; or, the Diagnostic Classification of Mental Health and Development Disorders of Infancy and Early Childhood Zero-to-Three. Criteria for these diagnoses can be ascertained by any of the above methods. Additionally, targeted clinical studies can employ laboratory procedures such as functional imaging (e.g., positron emission tomography scans or functional magnetic resonance imaging), electroencephalograms, or other techniques to measure brain function. While potentially powerful, those imaging modalities are not appropriate for inclusion in the core protocol of a broad-based longitudinal study. Consequently, the NCS will rely on a combination of screening instruments and diagnostic information, including records of health care provider diagnoses, to identify developmental and mental health disorders.

Specific conditions of interest to the NCS include: learning, sensory, and motor disabilities; autism-spectrum disorders; attention and conduct problems (e.g., ADHD); depression and anxiety disorders; and schizophrenia. Importantly, the NCS will not only attempt to capture conditions meeting diagnostic criteria, but will also use instruments that capture relevant symptoms in order to identify subclinical manifestations operating below diagnostic thresholds. Early identification of symptoms will be dependent primarily upon reliable and valid parental report screening tools. Direct diagnostic assessment of the child will be used whenever possible. Between birth and age 1, the conditions of primary concern to the NCS are sensory and motor disabilities, as well as serious developmental delays. Early screening for autism and for early precursors of mood and behavioral disorders will be added to these domains between ages 1 and 2.

Diagnoses will also be confirmed, whenever possible, through the child's medical records of documented clinical diagnoses by the child's pediatrician or other health care providers. The American Academy of Pediatrics (AAP) issued a policy statement in 2006 instructing child health care providers to engage in a program of developmental surveillance, screening, and diagnosis (Council on Children with Disabilities et al., 2006). Specifically, the AAP recommended that surveillance take place throughout infancy and early childhood, and that regular developmental screening tests be administered at well-child visits at 9, 18, and 30 months. Positive screens should then be followed by full diagnostic evaluations and referrals to early intervention. By obtaining confirmation through the child's medical records when possible, the NCS should be able to track provider diagnoses of developmental and behavioral disorders. Using a combination of screening instruments for symptoms and diagnoses from health care providers, the NCS will track not only the onset of neurodevelopmental, behavioral, and mental health symptoms and disorders, but the course of the disorders across development. Through repeated assessments over time, the NCS will be able to examine trajectories of children with diagnoses, including precursors of disorder and responses to early intervention efforts. This will permit a better understanding of the stability and course of disorder as children develop and are exposed both to treatment and to new and different environmental influences.

Assessment of learning, sensory, and motor functioning and disabilities

Some sensory and motor difficulties are evident very early in the child's life, and such disorders are usually more severe than those identified later. Other sensory and motor disorders can often be identified by age 2, whereas learning disabilities are often not identified until children enter school. Routine infant hearing screening is recorded in the hospital chart at birth, which will be abstracted by the NCS. Screening for sensory and motor disabilities on the NCS will begin before the neonate has been discharged from the hospital. During a pre-discharge examination, the infant's neurological status will be assessed using the NICU Network Neurobehavioral Scale (NNNS)(Lester & Tronick, 2005), a direct examination of neuromotor and neurobehavioral functioning. The NNNS is an effective screen for problems in early neurobehavioral functioning and has been shown to be sensitive to in utero substance exposure (Lester et al., 2002).

The NCS will continue to track children's developmental status during infancy with regard to cognitive, motor, and language delays using multiple assessment strategies. At 12 months, the NCS will administer three of the Bayley III Scales of Development: Cognitive, Motor, and Language (Bayley, 2006) to all enrolled children to assess the achievement of developmental milestones within these domains. The Bayley is a widely used and extensively normed assessment of developmental functioning long recognized as a standard in the field of developmental assessment (Albers & Grieve, 2007; Sattler, 2001). Low scores can be interpreted as indicating developmental delay (Bayley, 2006).

In addition to the administration of the Bayley III, actual diagnosis of learning, sensory, and motor disabilities will be confirmed whenever possible through the child's medical records, including the diagnoses and treatment plans of their medical providers. The child's health care visits will be reviewed at every contact with the parents, including both in-person contacts at 6 and 12 months and phone contacts at 3, 9, 18, and 24 months, and will continue to be assessed regularly after that. Throughout childhood and adolescence, the child's developmental status with regard to cognitive, language, and motor functioning will continue to be assessed periodically through direct testing by the NCS, and diagnoses confirmed whenever possible through health care provider diagnoses.

Assessment of autism-spectrum disorders

Autism-spectrum disorders, including but not limited to autism, Asperger's syndrome, and other pervasive developmental disorders, are not generally diagnosed until the child's second year or later (Robins & DuMont-Mathieu, 2006). Autism is a developmental disorder of great concern because of increased prevalence and unknown etiology in most cases. Symptoms related to the autism spectrum include deficits in social behavior and communication, and repetitive and stereotyped behavior, and often extend to cognitive impairments or motor abnormalities (Diagnostic and Statistical Manual of Mental Disorders IV-Text Revision, 2000). The NCS will begin screening for autism spectrum disorders when the child is 18 months old and continue to screen for symptoms periodically through the toddler and preschool period. The screening instrument the NCS will use is the Modified Checklist for Autism in Toddlers (M-CHAT)(Robins, Fein, Barton, & Green, 2001), a parental report instrument that has excellent psychometric properties. The M-CHAT, however, is a screen for risk of autism and does not yield a diagnosis of autism or autism spectrum disorders. For diagnostic information, the NCS will rely on diagnostic assessments conducted by the children's health care providers and abstracted from medical records whenever possible. This will include not only private pediatrician contacts, but also diagnoses received through specialty clinics for developmental disorders or through early intervention programs.

Assessment of depression, anxiety, and attention and conduct problems

Behavioral, attention, and mood disorders are rarely diagnosed in infants. Although infants display variability in their moods, conduct, and attentional abilities, configurations of individual differences in these domains are not usually sufficiently stable to warrant diagnoses at this age (Zeanah, Boris, & Scheeringa, 1997). Little is known, however, about precursors of adult mental illness, so during the infancy period the NCS will assess social and cognitive behaviors that may be precursor symptoms to later problems. At 12 months the parent will be asked to complete the Brief Infant-Toddler Social and Emotional Assessment (BITSEA)(Briggs-Gowan, Carter, Irwin, Wachtel, & Cicchetti, 2004) a validated screening instrument which assesses risk for mood problems, behavior problems, and self-regulatory deficits. The BITSEA, or an age-appropriate modification of the BITSEA, will be repeated through the toddler and preschool period to track risk for problems over time. As the children age, other similar screening instruments will be used, such as the well-validated and widely used Strengths and Difficulties Questionnaire (Bourdon, Goodman, Rae, Simpson, & Koretz, 2005; Goodman, 1997), which assesses conduct problems, emotional problems, hyperactivity and inattention problems, and relationship problems, and can be completed by parents, teachers, and in the teen years by the adolescents themselves.

Early diagnoses of disorders will be confirmed whenever possible through the children's health care providers' records. Later in childhood, measures and diagnostic interviews such as the Preschool Age Psychiatric Assessments (PAPA)(Egger & Angold, 2004) interview or the National Institute of Mental Health Diagnostic Interview Schedule for Children (NIMH-DISC-IV)(Shaffer, Fisher, Lucas, Dulcan, & Schwab-Stone, 2000) may be used to supplement diagnostic information from the

health care providers and assure diagnostic information on children who do not visit health care providers regularly.

Assessment of schizophrenia

Schizophrenia, a psychotic disorder believed to have both genetic and environmental etiology (Walker, Kestler, Bollini, & Hochman, 2004), will also be of interest to the NCS. Schizophrenia, however, is rarely diagnosed before late adolescence or early adulthood (Hafner, Maurer, Loffler, & Riecher-Rossler, 1993). Because screening and diagnostic procedures for schizophrenia will likely continue to evolve before the NCS children reach that life stage, no specific screening or diagnostic tool will be identified at this point. Instead, the best tools available at the time will be evaluated for use on the NCS.

8.2.3 Assessment of Related Factors

There are many exposures that have the potential to exacerbate or buffer the aspects of children's neurodevelopmental and behavioral functioning that will be assessed on the NCS. This includes exposures such as prenatal infection and inflammation, child and parental exposure to organophosphate pesticides, social relationships, and child and family engagement with social institutions such as child care, schools, and religious organizations. Environmental and social exposures will also be investigated as they interact with genetic factors, such as genetic alleles for paraoxonase-1 or variations in the 5-HTT genetic alleles, to produce risk or protective factors for child neurodevelopment and behavior outcomes. See Section 9.5 for details.

8.3 Child Health and Development

8.3.1 Definition

Identification of neurodevelopmental, behavioral, and mental health disorders, as discussed in Section 8.2, is essential to the mission of the NCS. Nonetheless, the tracking of normative developmental trajectories—normal growth in functioning across domains that occurs with age—is equally important. Although there is overlap in the domains of functioning between the outcomes of neurodevelopment and behavior and child health and development, there are important conceptual and practical distinctions that warrant the separate consideration of these outcomes. Child health and development is concerned not with disorder or symptoms of disorder, but with individual differences in trajectories of normal, healthy adaptation over time. Age-appropriate development in social, cognitive, and behavioral and emotional health domains is usually defined either through age-normed benchmarks from standardized testing, or identification of patterns of adaptive functioning in a particular domain. For example, cognitive abilities, temperament, and social competence can be assessed along a continuum and tracked longitudinally among all children participating in the NCS. Details of child health and development measures appear in Appendix F.1.

8.3.2 Assessment of Developmental Trajectories

One challenge in assessing developmental trajectories is that behaviors indicative of normal development at one age—clinging to a caregiver who is trying to leave, for example—may be a marker of maladaptation later in development (Sroufe, 1979; Sroufe & Waters, 1977). At each phase of

development, decisions must be made regarding the most appropriate assessment tool to capture the construct of interest and close attention must be paid to interpreting the information in a developmentally appropriate fashion. A great deal of effort has been expended to find efficient, valid, and reliable assessments that will enable effective measurement of the same domains across developmental stages. The NCS will assess multiple domains of child development using standardized and frequently used instruments. One criterion for these assessments is their ability to connect to later developmental measures of the same constructs. Areas to be covered include cognitive processes, language, and social and emotional development.

Assessment of development and behavior in children is challenging, whether in clinical or research settings. For younger children, in particular, reports of child functioning are often dependent upon subjective parental or other third party reports, which can lead to reporting biases (Fiske, 1987). Determining the best assessment modality for child functioning requires careful planning and consideration. In addition, administration of direct assessments to a child requires attention to the child's comfort in the testing situation, whether at home or in a clinical setting, as variations in feelings of ease or distress can affect children's responsiveness to the examiner and cause dramatic variations in testing outcomes. All examiners who administer assessments to children on the NCS will be extensively trained in developing rapport with children.

Assessment of cognitive and language abilities

Trajectories of cognitive and language development are important indices of developmental progress and problems as the child's experiences and exposures can compromise healthy trajectories of cognitive and language development (Cicchetti, Rogosch, & Toth, 2000). The NCS will begin tracking these milestones during infancy and continue tracking them through adolescence. At 12 months, the NCS will administer the cognitive and language subtests of the Bayley III Scales of Development (Bayley, 2006), a direct child assessment of achievement of developmental milestones within these domains. The Bayley is a widely used and broadly normed "gold standard" assessment of developmental functioning (Albers & Grieve, 2007; Sattler, 2001). The cognitive subtest assesses sensorimotor development, exploration and manipulation, object relatedness, concept formation, memory, play, and other aspects of cognitive processing. The language subtest includes both expressive and receptive language ability. Also at 12 months the child's parent will be asked to complete the MacArthur-Bates Communicative Development Inventory Short Form (CDI)(Fenson et al., 2000). The CDI was developed to supplement direct assessment of child language by obtaining information about the parent's broad knowledge of the child's communication skills, thus incorporating information more representative of children's everyday language use than what can be obtained in a relatively short direct assessment. Both the vocabulary checklist and the actions and gestures communication subtests of the CDI will be administered.

During the preschool years and beyond, measures of cognitive and language abilities will reassess similar constructs, such as concept formation, memory, and expressive and receptive language ability. This may include repeating the Bayley III scales, or at older ages using developmentally appropriate standardized assessments of achievement and intelligence such as the Woodcock-Johnson Test of Achievement (McGrew & Woodcock, 2001) and the Kaufman Brief Intelligence Test (Kaufman & Kaufman, 2004), both of which will also be used to assess parental abilities. This will permit an examination of the ways in which cognitive abilities and achievement can change over time, as the child responds to experiences and exposures in the environment. Other assessments will focus on additional developmentally relevant abilities such as executive function and attention, and will use a combination of parent and teacher report and direct testing of the child.

Assessment of social and emotional development

Social and emotional development covers several important domains of child functioning, both intrapersonal and interpersonal. Infants begin this trajectory with basic temperamental qualities and with the formation of their first relationships: parent-child relationships. They face subsequent challenges in learning to regulate their emotions and behavior and to navigate the increasingly complex reciprocity of relationship interactions. Social and emotional development in the first two years will be assessed on the NCS through a combination of parental report and direct observation.

Assessing temperament early in development is important, as temperamental qualities not only exert direct influence on children's adjustment but also influence parental reactions to the infant's signals and needs and thus affect subsequent development indirectly. When the infant is 6 months old, the NCS will collect maternal reports of child temperament using three subscales of the Rothbart Infant Behavior Questionnaire-Revised (IBQ-R)(Gartstein & Rothbart, 2003; Rothbart, 1981), including activity level, fearfulness, and positive anticipation of and approach to novelty. The IBQ-R asks the parent to report on specific, recent infant behaviors, a technique that minimizes parental bias in the report of child temperament (Rothbart & Goldsmith, 1985).

Also at 6 months, the NCS will conduct its first videotaped observation of mother-child interaction. This will entail videotaping the mother and infant for 15 minutes as they engage in a semistructured play session with a set of toys provided for them during the visit. This technique has been used on many studies, including the NICHD Study of Early Child Care and Youth Development, and the associated coding scheme taps elements of parent-child interaction such as parental sensitivity and cognitive stimulation (National Institute of Child Health and Human Development Early Child Care Research Network, 2003). Observation is considered the "gold standard" of assessment in the domain of parenting (Zaslow et al., 2006).

At 12 months the child's social and emotional development will be assessed using parental report on the BITSEA (Briggs-Gowan et al., 2004). The BITSEA, a well-established brief measure of infant and toddler problems and strengths, assesses effective behavioral, and emotional self-regulation as well as social competence skills, including compliance, attention, mastery motivation, imitation/play, empathy, and prosocial peer relations. At the 12-month visit, a parent-child interaction observation will be repeated, but this time with the child and the child's alternate caregiver (it is anticipated that this will most often be the child's father), giving an expanded view of the child's functioning within the social network.

During the toddler and preschool years, the same constructs will be assessed again, using the same procedures where appropriate, or assessments that are age-appropriate measures of these constructs such as the Strengths and Difficulties Questionnaire (Bourdon et al., 2005; Goodman, 1997), which assesses prosocial behavior and relationship skills.

As the child ages and begins to spend time in the broader social contexts of school and community, assessments of developmental trajectories in social and emotional competence will be tailored to include these normative changes. This will involve assessing family, peer, and eventually romantic relationship qualities in addition to child functioning and adaptation across multiple contexts, such as home and school. It is anticipated that although parents are the primary reporters on child behavior in infancy and early childhood, eventually both teachers and the children themselves will serve as respondents. It is also anticipated that direct observation of parent-child interaction will continue to be assessed periodically throughout development.

8.3.3 Assessment of Related Factors

Numerous factors may influence a child's developmental and behavioral health trajectories. Prenatal exposures, such as exposure to tobacco, alcohol and maternal infection, that potentially have broad health effects will be collected through a variety of mechanisms. Ascertainment of prenatal and postnatal exposure to environmental chemicals with potential impact on the child's developmental trajectories is also outlined in Section 9.1

Questionnaire data will be used to ascertain parental cognitive function and literacy (e.g., Kaufman Brief Intelligence Test; and Woodcock-Johnson III Letter-Word Identification and Passage Comprehension subscales), maternal depression during pregnancy and several times after delivery (Center for Epidemiological Studies Depression Scale), family history of psychiatric diagnoses, and measures of family process and parenting style. Information concerning child care environments and, later in life, school environments will also be collected through maternal questionnaire, direct observation in child care settings, or interview of providers. Information collected on prescription medication use and from the health diary may also be used as sources of conditions of interest in either the mother or the child.

8.4 Asthma

8.4.1 Definition

Asthma is a complex respiratory disease characterized by episodic, reversible, inflammation-mediated constriction of small and large airways. The resulting airway obstruction leads to air trapping and clinical manifestation of the disease: wheezing, dyspnea, and hypoxia. Severe untreated attacks can be fatal. Data from gene association studies indicate a complex inheritance pattern involving perhaps hundreds of genes governing the expression of varying asthma and atopy phenotypes (Ober & Hoffjan, 2006). Asthma phenotypes that emerge from the first through sixth year of life have been predictive of persistent asthma symptoms and long-lasting decrements in lung function in cohort studies (Stein & Martinez, 2004). Childhood "asthma" can be categorized into three phenotypes: (1) airway obstruction which begins in the first two years of life but does not persist to school age, often referred to as early onset transient airway obstruction; (2) early-onset airway obstruction that persists past school age, or early-onset persistent asthma; and (3) recurrent airway obstruction that begins after the first few years of life, or late-onset asthma (Martinez & Helms, 1998; Stein & Martinez, 2004). Prospective data are needed to examine risk factors for the development of these phenotypes and for persistence of early airway obstruction into later childhood and adulthood.

8.4.2 Assessment of Asthma

The NCS will be able to assess the effect of timing of exposures, particularly during critical windows of vulnerability (e.g., specific trimester of pregnancy, early vs. later postnatal periods, etc.), on the development of childhood asthma. This will include distinguishing the effects and interactions of biologics, air pollutants, and genetics with other potential causative factors (e.g., social and economic status, health care access, diet, stress). Accurate exposure and phenotypic data are needed to assess the significance of various asthma and allergy genotype-complex exposure interactions that result in several different asthma phenotypes (Bel, 2004; Taussig et al., 2003). Identification of children at risk for developing severe forms of asthma would have clear public health impact.

Diagnosis

There is no single test that provides an unequivocal diagnosis of asthma. In young children, the clinical diagnosis of asthma is based on relevant history and pulmonary auscultation. Chest x-ray and pulse oximetry are often used in the initial diagnosis and to monitor disease. When the child is old enough to cooperate, approximately 5 to 7 years old, spirometry or more detailed pulmonary function tests can be used for objective assessment of pulmonary status, though this is rarely necessary for clinical diagnosis. Spirometry or peak flow monitoring can be used to follow disease status and progression.

In population-based research, diagnosis of asthma generally is based on combinations of reported symptom history, reports of physician-diagnosed disease, and medical records. Clinical research studies use pulmonary function tests, often in combination with provocation tests such as methacholine or exercise challenges, to attempt to obtain objective measures of lung function. Recent advances in passive pulmonary function assessment have enabled those measures to be obtained in children as young as several days old; however, the equipment is expensive, results are operator dependant, and the procedures would most likely not meet the "minimal risk" criterion.

Assessment of asthma in the NCS

Information on symptoms, signs, and other factors related to asthma will be collected throughout the course of the NCS using multiple methodologies. Questionnaires will assess a child's history of asthma symptoms (using questions based on the International Study of Asthma and Allergies in Childhood) starting with the six-month visit and continuing through adolescence. Questions regarding recent or "ever" diagnosis of asthma in a medical setting will also be asked, and information on the use of asthma-related medications will be collected. Confirmation of physician diagnoses related to any office visits, emergency department visits, and hospitalizations will be obtained whenever possible. Attempts to measure lung function via spirometry will begin at the 36-month clinic visit, although the difficulty of obtaining consistent results at this age is recognized. These measures will be repeated at subsequent clinic visits. In addition, the NCS may ask for periodic peak flow measures to be taken at home and entered into the child's health care visit log beginning at approximately age 5. NCS field staff and medical professionals will assess allergic sensitization and allergic and nonallergic rhinitis and asthma. Immune system function can also be evaluated through such measures as lymphocytes, cytokines, IgE, and interlukins. This approach of multi-method, repeated measures of asthma over time will permit investigation of the ways in which symptoms are ameliorated or exacerbated as the child faces new environmental exposures and attempts new treatment strategies.

The NCS will collect biological samples that could be the source of DNA for traditional genetic analysis of candidate genes and for gene discovery based on genome-wide association scans. The anticipation of chip-based genotyping of all participants based on current technology, or complete sequencing of all individuals at some point in the future, will provide extraordinary detail about genetic variation of nuclear DNA. Along with the planned definition of cases based on asthma phenotypes, this provides an opportunity to use the efficient nested-case control design for subsets of the sample, or the proportional hazard design for the entire sample, to evaluate effects of genes, environments, and their interaction. In addition, the Study will collect biological samples at multiple points over time, which provides the opportunity to evaluate epigenetic changes proposed to be the molecular mechanisms of some gene-environment effects (Hanson & Gluckman, 2005). The epigenetic assays for environmental effects of methylation and chromatin status are rapidly evolving, and current methods are sure to improve rapidly as a result of extensive current work (Callinan & Feinberg, 2006) and anticipated future work.

Two overlapping study populations will be used to address asthma hypotheses. For questionnaire items and other data available for the entire cohort, we will analyze the full NCS sample data. For genetic data and other items that require laboratory processing, the study population will consist of a nested case-control study design with sampled cases such as those with wheezing/asthma symptoms and a matched cohort of controls. Matching factors might include date of birth, regional location of birth, race, gender, and socioeconomic status.

Assessment of upper and lower airway disorders

It should be noted that with disorders associated with the upper and lower airways, there exist numerous definitions that can be employed at different developmental stages depending on the availability of subjective (parental report) vs. objective (spirometry, sensitization) criteria. Below are potential outcomes that may be used in the NCS at different child ages depending upon data available at each interview or clinical exam.

- *IgE antibody quantification*: Total and allergen-specific IgE antibodies (RAST) can be measured from serum levels. One advantage of the RAST test is that it does not have to be performed in a physician/hospital office and can be done instead at the child's home. The disadvantage is that it requires a venipuncture and therefore may be less acceptable to children and their primary caregivers. Sensitization status can be ascertained by the measurement of specific IgE to mite, cat, dog, grasses, foods, etc., in serum levels of infants and children (Simpson et al., 2005).

- *Rhinitis:* Rhinitis presents with a constellation of symptoms, including: nasal congestion; sneezing; rhinorrhea; itchy nose, mouth, throat and/or ears; itchy, watery, and red eyes. Symptoms are present without a cold and last for a minimum of one month (American Academy of Allergy, Asthma, & Immunology, 2005).

- *Wheeze:* Diagnosis of wheeze symptoms consistent with the airway obstruction associated with asthma is dependent on a clinical history reported by a parent, and may not be predictive of current or future development of childhood asthma. Wheezing is likely to be the primary outcome until the child can be tested using objective measures such as pulmonary function testing. Wheezing can be defined as an episode of wheezing or whistling in the chest without a cold for a certain number of times over a specified time period. The presence of wheeze without a cold is a common definition used to identify potential asthma in young children. There are additional related questions for defining more serious wheezing in the young child, such as: total number of attacks; if the child's sleep is disturbed with wheezing; if the child sounds wheezy after exercising; and if the child has a dry cough apart from a cold. Inquiry into these symptoms will be standardized.

- *Asthma:* Diagnosing asthma in young children can be difficult, and under- or over-diagnosis is a problem. As the child ages, a diagnosis of asthma may be confirmed by pre- to post-bronchodilator forced expiratory volume in one second (FEV_1) in children who meet certain predetermined criteria such as: wheezing symptoms in the previous 12 months; physician treatment for "asthma" in the previous 12 months; or an increased exhaled nitrous oxide (eNO) of greater than 10 parts per billion. Confirmatory tests would allow for additional outcome definitions to be assessed, such as allergic asthma disease, nonallergic asthma disease, allergic asymptomatic airway reactivity (AR), and nonallergic asymptomatic AR.

- *Eczema:* Despite the fact that eczema is not an airway disease, the NCS may examine atopic dermatitis as an outcome since it is one of the earliest allergic diseases in childhood, and it is associated with asthma. The clinical criteria for childhood eczema can be determined by the parent questionnaire and/or a physical examination.

8.4.3 Assessment of Related Factors

In addition to measures related to ascertaining asthma in the child, relevant information regarding risk and exposures will be collected. For example, family history of asthma and atopy will be obtained via the T1 questionnaire. We will examine maternal psychosocial stress during pregnancy, including stress life events, social isolation, racism/discrimination, anxiety, depression, cortisol in saliva, urinary catecholamines, and low socioeconomic status. Other potential gestational factors are prematurity and the child's birth weight. History of early life infections will be collected through the child's health care visit log as well as by the six-month questionnaire. The relation between the risk of developing early onset transient airway obstruction and the diet during pregnancy and early childhood will be explored. The dietary variables include consumption level of antioxidant and other micronutrients, fresh fruits and vegetables, and vitamin intake. Other dietary factors of interest include breast feeding and its relation to the risk of early onset persistent wheezing, and obesity and its relation to the risk of late onset asthma. Indicators of socioeconomic status include household income, educational level, location of residence, household composition, housing characteristics, neighborhood and community characteristics (age, race/ethnic composition, population density, housing quality), neighborhood resources (community organizations, schools, recreational facilities, public services, commercial outlets, religious organizations), and neighborhood processes (neighborhood cohesiveness, crime levels, political activity, police activity, family's perceptions of neighborhood). These factors can be associated with exposure to social, physical, psychological, and environmental factors related to the risk of asthma. Exposure to allergens early in life will be measured through dust samples as well as specific questionnaire items (e.g., presence of pets). Air pollution data will be obtained by questionnaire or biomarker (e.g., tobacco), use of ambient air pollution monitors, and home-based air quality measurements at periodic home visits. Child care and school exposures will also be obtained by the NCS, either by direct measurement, when possible, or by other sources, as necessary.

8.5 Obesity, Body Composition, and Growth

8.5.1 Definition

The ongoing increase in childhood obesity and overweight in the United States gives rise to numerous questions regarding both the antecedents of overweight and adiposity and their long-term health effects (Flegal & Troiano, 2000; Ogden, Fryer, Carroll, & Flegal, 2004; Ogden et al., 2006). Obesity appears to remain consistent from childhood into adulthood (Serdula et al., 1993), and childhood obesity is directly related to the same adverse health outcomes generally associated with adult obesity (Freedman, Khan, Dietz, Srinivasan, & Berenson, 2001; Haji et al., 2006; Orio et al., 2007). Although the cause of overweight in an individual is ostensibly obvious—energy intake greater than expenditure—elucidation of reasons behind the population-level trends in obesity and overweight is necessary to enable appropriate interventions.

Most frequently, obesity is defined simply in terms of the relation of weight to height. For example, children who are ages 2 through 19 years are considered above the range of a healthy weight if their body mass index (BMI, an index of weight in relation to height) is above the 85th percentile compared to other children in their age and gender group (Centers for Disease Control, 2007). For adults, a BMI of greater than 25 is considered above the range of a healthy weight. BMI is correlated with overall

body fat and directly associated with adverse outcomes. However, it is neither a true measure of adiposity nor a measure of the relative distribution of central (visceral) and peripheral fat, characteristics that may be the true risk factors for adverse outcomes (Arner, 1998; Bergman et al., 2006). To the extent possible within the overall structure of the diverse Study protocol, the NCS will strive to obtain measures of obesity, body composition, and growth that go beyond simple relations between weight and height.

8.5.2 Assessment of Overweight and Obesity

Maternal and paternal measures

Assessment of maternal size and body composition will start with the first home visit, either before pregnancy or in the first trimester of pregnancy and continue throughout pregnancy until birth. Initial measurements will include height, weight, waist and hip circumferences, and triceps and subscapular skin folds. Segmental heights (e.g., knee height) will be obtained as there are established associations between those measures, prepubertal (and possibly intra-uterine) nutrition and growth, and later cardiovascular outcome (Gunnell et al., 2003). The subscapular-triceps skinfold ratio is among the commonly used estimates of fat distribution, often used in combination with the waist-to-hip ratio and BMI (Stein et al., 2007). Similar measures will be obtained from the father during the first trimester home visit.

Weight and skinfolds will be obtained from the mother at each subsequent pregnancy visit, because of potential associations between maternal weight gain and adiposity, in utero growth, and subsequent metabolic and cardiovascular outcomes (Barker, 1992). The assessment of maternal body composition by anthropometry during pregnancy is complicated by the attendant changes in body water, including skin and subcutaneous accumulation (Huston Presley, Wong, Roman, Amini, & Catalano, 2000; Stevens-Simon, Thureen, Barrett, & Stamm, 2001). Skinfold measures during pregnancy, however, are often used and remain a reasonable choice within the context of the NCS and the focus on subsequent child growth and health.

Child measures

In the NCS, assessment of the child's body habitus and composition will begin in utero with the second and third trimester ultrasounds. Along with routine linear measures of growth, measures of mid-thigh lean and fat mass area and abdominal wall thickness will be obtained (Bernstein & Catalano, 1991; Bernstein, Goran, Amini, & Catalano, 1997). Though not generally part of a routine fetal ultrasound, those measures are used in a growing number of studies, can be obtained with reasonable accuracy and precision, and will provide fetal analogues to the postnatal anthropometric estimates of peripheral and central fat distribution described below.

At birth, anthropometric measures will include weight, length, head circumference, body segment lengths, and triceps and subscapular skinfolds. These measures will be repeated at each home visit, until the three-year clinic visit. At that time, and at later clinic visits, DXA and BIA will be attempted, in concert with the ongoing anthropometric measurements. Depending on their availability during the Study period, other measures of body composition, such as air displacement plethysmography (Winsley et al., 2005) or MRI (Pietrobelli, Malavolti, Fuiano, & Faith, 2007) could be used on all or on a subset of the NCS population.

The correlation between the anthropometric and other body composition measures is variable and depends on the age of the participant, the measures used, the participant's body habitus, and

perhaps even gender (Semiz, Ozgoren, & Sabir, 2007; Winsley et al., 2005; Wright et al., 2007). However, there is evidence that, at least later in childhood, the anthropometric measures do correlate with metabolic measures of interest to the NCS, providing confidence in the use of those measures as a cornerstone of body composition assessment (Freedman, Serdula, Srinivasan, & Berenson, 1999).

Interpretation of measures of growth and body composition

An individual's growth can be quantified two ways. The first is by comparison with published references, such as the CDC BMI and BMI percentile curves (http://www.cdc.gov/nccdphp/dnpa/bmi/childrens_BMI/about_childrens_BMI.htm). However, population references do not exist for many of the measures the Study will use. In those instances, and even when external references do exist, internal NCS distributions can also be created and used. The probability-based sample of the NCS should provide a strong basis for quantification and, perhaps, serve as an external reference for future studies. Skin fold thicknesses can be analyzed as absolute measures, as ratios, or as terms in any of a number of equations that can be used to estimate percentage body fat. The NCS will report the absolute measurements, not the results of estimating equations, giving the analyst maximal flexibility in the definition of obesity or body composition.

8.5.3 Assessment of Related Factors

A child's energy expenditure early in life will be assessed primarily through parental report of activity and activity diary completion. In late childhood and in the assessment of parental activity, questions will be based on the International Physical Activity Questionnaire (IPAQ), both to enable comparison with other studies and to permit the estimated quantification of metabolic equivalence of task (MET) expenditure. Though the use of the IPAQ in adults is well validated (Craig et al., 2003), its performance among adolescents is less predictable and may vary depending on the study population (Arvidsson, Slinde, & Hulthen, 2005; Craig et al., 2003). As the NCS progresses, additional measures of physical activity, either questionnaire-based or activity-based (e.g., accelerometers), may be employed.

Fetal exposure to maternal glucose will be estimated by several HgbA1c measurements during pregnancy, as discussed in Section 9.4.3. Fasting blood samples to be analyzed for glucose, insulin, and lipids will be collected from at least some of the women during the third trimester clinic visit. Maternal diet will be assessed throughout pregnancy and after birth through a combination of food frequency questionnaires, food diaries, and recalls. Infant feeding practices, including breast and bottle feeding, and subsequent child diet will be collected via questionnaire. Family process, physical activity, and the child's exposure to television and other media will also be collected by questionnaire early in life. Neighborhood characteristics that may be conducive to physical activity, or lack thereof, will be assessed by parental report and by community-level observations and data. See Chapter 9 for details on these measures.

Potentially relevant laboratory analyses can include assessment of ghrelin, leptin, adiponectin, and other "adipocytokines" to determine if those compounds are causally related to increased weight and adiposity or are an intermediate phenotype. These can be measured in maternal blood as well as in the child from infancy onward. When the child is older, it may be possible to obtain fasting blood tests for assessment of glucose and insulin, although that will be determined at a later point in the study. Lipid profiles can be assessed starting with 12 month visit, though fasting samples are necessary for triglyceride assessment.

8.6 Injury

8.6.1 Definition

Injuries are a leading cause of childhood death and disability. After age 1, they are the single leading cause of death among children and adolescents in the United States. Injuries are generally classified by both the external cause (e.g., car crash, poisoning, fall) and the intent. Of the 23,636 injury deaths to U.S. children (ages 0-21 years) in 2004, 15,871 (67 percent) were unintentional ("accidents") (Centers for Disease Control and Prevention, National Center for Injury Prevention and Control [CDC NCIPC], 2007). Intentional injuries (interpersonal violence, child maltreatment, and self-inflicted injuries) and injuries of undetermined intent accounted for the remaining 7,765 fatalities. The leading causes of injuries vary with the age of the child, with intentional injuries taking their greatest toll in adolescence (CDC NCIPC, 2007).

The World Health Organization injury surveillance guidelines (Holder et al., 2001) define injury according to the guidelines of Baker, O'Neill, Ginsburg, and Guohua (1992): "Injuries are caused by acute exposure to physical agents such as mechanical energy, heat, electricity, chemicals, and ionizing radiation interacting with the body in amounts or at rates that exceed the threshold of human tolerance…In some cases (for example, drowning and frostbite), injuries result from the sudden lack of essential agents such as oxygen or heat." (Baker et al., 1992, p.4). According to this definition, an injury from a motor vehicle crash would be due to exposure to mechanical energy, a scald burn due to exposure to thermal energy, and a poisoning due to exposure to a chemical agent.

It is expected that, of the 100,000 children enrolled in the NCS, more than 1,600 children will die from an injury and more than 8,000 will be hospitalized (Rivara & Villaveces, 2001) with many more seeking care from an emergency department or other health care provider. Virtually all children experience minor injuries (e.g., cuts and bruises). A challenge for the NCS is to use a definition that identifies the subset of injuries that are serious enough to potentially compromise health and future development. Although many studies include only those injuries for which medical care is sought, this definition is biased in a way that would preferentially identify injuries among those with greater access to health care. Thus, comparable to definitions used in the International Study of Health Behavior in School Children (Currie et al., 2004; Molcho et al., 2006; Pickett et al., 2005; Pickett et al., 2006), in the NCS an injury will be defined as physical damage to an individual that results in medical care or at least one day of limitations in activity.

8.6.2 Assessment of Injury

Beginning in early life and continuing through early childhood, ascertainment of injuries will be based on parental report. Later in childhood and adolescence parent or caregiver reports will be supplemented with self-reports from the child. Health visit logs will be provided to study participants for documentation of medical visits and will include a place for documentation of key data elements about injuries that result in medical care. Activity diaries may help identify injuries that result in limitations in activity, and specific activities associated with increased risk of injury.

Studies have shown that recall for injury events declines with time and severity of the injury (Cummings, Rivara, Thompson, & Reid, 2005; Harel et al., 1994). In one study, approximately 3 months following the event, parents were able to recall 88 percent of major injuries, 86 percent of minor injuries seen in an emergency department, 81 percent of minor injuries seen in an urgent care setting, and 58 percent of minor injuries seen in a clinic. At 6 months, recall of major injuries was 80 percent, but dropped to 56 percent by one year (Cummings et al., 2005). Throughout the Study, parents, caregivers,

and, when appropriate, children will be asked about injuries that have occurred during the interval period. Thus, from birth through age 1, information about injuries will be ascertained every 3 months and from 1 through 3 years every 6 months. Contact periods for later years have not yet been determined. For those reporting an injury, additional information will be sought about the external cause of the injury, the physical harm to the child (i.e. the nature of the injury), treatment received, and any lasting sequelae.

Traumatic brain injury is of particular interest to the Study both as a primary Study outcome and as a confounder with significant potential to impact trajectories of development and thus multiple other Study outcomes. Consequently, when a head injury is reported additional questions will be asked about changes in level of consciousness. Although even minor traumatic head injury can be identified through diffusion MRI or other advanced structural and functional imaging technologies (Suh, Davis, Hopkins, Fajman, & Mapstone, 2001), the expense and participant burden of such technologies are not appropriate for general use in the NCS population. At this time, biomarkers for measurement of head injury are not available for use in an epidemiologic study. However, it is recognized that this is an area of active research that may have implications for future protocol development or use of stored biospecimens (Berger et al., 2006; Berger & Kochanek, 2006).

Methods are being explored to supplement the self-reported data with data collection from medical records for the most severe injuries. This effort will likely be limited to those injuries that result in hospitalization or death. Like self-report data, data abstracted from medical records would include information about the external cause of the injury, the nature of the injury, treatment received, and any lasting sequelae.

8.6.3 Assessment of Related Factors

Numerous factors may be related to child behavior that increases subsequent risk of injury. Areas of interest include prenatal and early life exposures to neurotoxic chemicals, including metals and organic pesticides, family process and parenting behaviors, and home, child care, and school environments (see Chapter 9). Examination of the genetic contribution to aggressive behavior, and thus risk of injury, will be examined both for direct genetic influence on behavior, and for the interactive effects of specific environmental exposures (e.g., chemical, socio-emotional) and genotype on aggressive behavior.

Factors with potential direct relation to injury risk, in addition to temperament and activity, include the physical characteristics of a child's home and neighborhood environments. These will be assessed by questionnaire and by direct observation by Study personnel. Medication use by the child may provide additional information on either conditions associated with injuries (e.g., seizure disorder, ADHD) or medications that might have a direct influence on risk of injury (e.g., sedating antihistamines).

8.7 Reproductive Development

8.7.1 Definition

Development of the reproductive system begins early in gestation and continues through infancy, childhood, adolescence, and into adulthood. A number of adverse outcomes can occur as the result of interference with development of this complex system, which includes the reproductive organs, the endocrine system, and the hypothalamic-pituitary-gonadal and hypothalamic-pituitary-adrenal axes that control their development and function. Early outcomes include birth defects such as hypospadias and cryptorchidism in boys (Pohl et al., 2007), as well as hormonal changes, such as hypothyroidism, in

boys and girls which interfere with optimal reproductive health. Later outcomes from the same exposures may include alterations in growth, timing, and progression of puberty (Herman-Giddens, 2006), and disease states such as polycystic ovary disease (PCOS) (Azziz et al., 2004) and endometriosis (Eskenazi et al., 2001) in females, and testicular dysgenesis syndrome in males (Skakkebaek et al., 2001).

Such changes in reproductive structure and development may be cumulative, that is, adverse outcomes at early ages may predispose an individual to be at greater risk for additional adverse effects, e.g., cryptorchidism and later changes in fertility (Lee, 2005) or early menarche and breast cancer (Vihko & Apter, 1986). In addition, recent studies in animal models suggest that certain exposures are associated with adverse reproductive development outcomes that are transgenerational, that is, effects that are carried into subsequent generations because of changes in DNA methylation patterns that are transmitted in the male germline to the next generation (Anway, Cupp, Uzumcu, & Skinner, 2005; Chang. Anway, Rekow, & Skinner , 2006).

8.7.2 Assessment of Reproductive Development

Evaluation of children at birth, at six month intervals during the first two years of life, and at regular intervals beyond that time, will allow assessment of birth defects and anthropometric measures. Health outcomes related to reproductive development can be assessed in relation to the timing of anthropometric measures and the physiologic development of reproductive organs and other regions of the body that respond to reproductive hormones. The NCS protocol will include physical examination for genital development and, where possible, will use medical record review for information on further diagnosis and/or surgical intervention. Following standard anthropometric measurement of weight, height/length, head circumference, and skin-fold thickness, a detailed observation of the body, particularly of the breasts and genitals, will be performed. Birth defects, such as hypospadias and cryptorchidism in boys or altered genital formation in girls, can be assessed at birth by direct observation by a medical professional or via medical record review when possible. Cryptorchidism (undescended testes) is determined by palpating for the testes in the scrotum. Surgical procedures may be required to fully diagnose and/or repair undescended testes. Hypospadias (abnormal opening of the urethal meatus along the ventral aspect of the penis) is diagnosed by direct observation. Location and severity of the urethral opening will be noted, and surgical repair may reveal more detailed diagnosis. Anogenital distance may also be measured at birth (Swan et al., 2005).

Measures of puberty onset (e.g., onset of breast development in girls, genital growth in boys, and pubic hair development in boys and girls), and stage of sexual maturation can be assessed used Tanner scales by self-assessment or examination by a medical professional (Marshall & Tanner, 1969; 1970). Validation of the best procedures to use for this assessment are likely to advance by the time children in the NCS reach age 6 or 7. Other key outcomes are age of menarche in females and spermarche in males. Menarche can be assessed through questionnaire or medical record abstraction, when possible, while spermarche can be determined through questionnaire and by examining for sperm in urine samples (Nielsen et al., 1986). In addition, hormone levels in both males and females obtained from blood samples throughout childhood will allow for the assessment of hormonal, thyroid, and pituitary gland status and function. It may also be possible to collect semen samples from participating male children when they reach age 18 to assess semen quality, sperm production, and morphology.

Serial assessment of reproductive outcomes at birth, in childhood, at puberty, and in adulthood, and collection of information from medical records, whenever possible, on disease states such as polycystic ovary disease (PCOS) and endometriosis in females and testicular dysgenesis syndrome in males, can be applied successfully to study reproductive development in the NCS. In addition, collection

of maternal breast milk and maternal and child blood and urine samples at multiple time points and serial questionnaires to assess pathways of exposure will provide the necessary data to evaluate both exposures and the links between exposures and reproductive development.

8.7.3 Assessment of Related Factors

Exposure to environmental agents that are hormonally active agents (HAAs; also called endocrine disruptors) has been shown to affect the reproductive system in a number of ways in both animals and humans. A variety of environmental chemicals have been cited in the literature as potential HAAs, including insecticides and herbicides (e.g., DDT, atrazine); pharmaceuticals (drug estrogens); chemicals associated with consumer goods/household products (e.g., bisphenol A, phthalates, nonylphenol, polybrominated diphenyl ethers [PBDEs], perfluorinated compounds [PFOA, PFOS]); industrial chemicals (e.g., polychlorinated biphenyls [PCBs], dioxins, polycyclic aromatic hydrocarbons [PAHs]); heavy metals (e.g., arsenic, lead, mercury, and cadmium); and natural hormones such as the phytoestrogens (Ashby, Tinwell, Stevens, Pastoor, & Breckenridge, 2002; Ceccatelli, Faass, Schlumpf, & Lichtensteiger, 2006; Eriksson, Fischer, & Fredriksson, 2006; Fenton, Hamm, Birnbaum, & Youngblood, 2002; Gray, Ostby, Cooper, & Kelce, 1999; Howdeshell, Hotchkiss, Thayer, Vandenbergh, & vom Saal, 1999; Kuriyama, Talsness, Grote & Chahoud, 2005; Lilienthal et al., 2006; McDonald, 2005; Rubin, et al., 2001; Schonfelder et al., 2002; Talsness et al., 2005; Wolf et al., 1999). Recent studies of environmental agents suggest that PCBs (Blanck et al., 2000) or organochlorine pesticides (Krstevska-Konstantinova et al., 2001) may accelerate pubertal development in girls while PAHs (Den Hond et al., 2002) or lead (Selevan et al., 2003; Wu et al., 2003) have been associated with delays in pubertal development. Data on the effects of HAAs on age at puberty in boys are fewer (Den Hond et al., 2002) but indicate an association between PCB and polychlorinated dibenzofuran (PCDF) exposures with delayed puberty and decreased penile length (Den Hond & Schoeters, 2006). These observations are concordant with laboratory data on the effects of HAAs. Because there are only limited data on specific critical windows for chemical exposures in relation to timing of puberty, the entire prepubertal period, including in utero growth and development and the peripubertal period, should be considered critical times for exposures. Environmental samples and biological specimens will be collected to allow measurements for a number of chemical exposures. These exposures will then be examined to look for associations with alterations in reproductive development.

Obesity, diet, and nutrition measures are important related factors for reproductive development. Higher percentage of body fat increases the risk of precocious puberty; later onset in underdeveloped nations is often attributed to poor nutrition (Anderson, Dallal, & Must, 2003). In addition, obesity and precocious puberty have been associated with conditions such as neurofibromatosis, hypothyroidism, PCOS, etc. (Cesario & Hughes, 2007). Delayed puberty has been associated with several conditions such as sickle cell disease, thalassaemia, Celiac disease, Gaucher disease type I, Cushing's disease, and other endocrine deficiencies. Anthropometric data, hormonal changes, and medical record abstraction, when possible, will be included in the NCS to examine these potential relations.

The prenatal and postnatal smoking status of parents may reduce the age of onset of puberty (Windham et al., 2004). Urine cotinine will be measured to examine active/passive smoking exposures.

Generally the mother's menstrual history is considered the biggest predictor of age of puberty, and some of this effect may be seen in ethnic differences (Blanck et al., 2000). There are genetic components for hypospadias, cryptorchidism, spermarche, and semen quality that may be related to the father's reproductive history (Pohl et al., 2007). In addition, genetic factors such as 5-alphareductase type 2 gene mutations (Silver & Russell, 1999) and androgen receptor mutations (Silver, 2000) are risk factors

for hypospadias. Maternal reproductive history and, when possible, paternal reproductive history will be collected using questionnaires and possibly medical records. Blood samples will be collected to determine genetic factors that may be involved.

There is some evidence that a younger gestational age at birth is associated with greater incidence of hypospadias and cryptorchidism (Pohl et al., 2007) and is a predictor of an earlier age at menarche; however, evidence points to small-for-gestational-age as the predictor of precocious puberty (Adair, 2001). Gestational age at birth and growth and development will be recorded in the NCS.

Maternal alcohol consumption may (Carbone et al., 2007) or may not (Blanck et al., 2000) be related to an increase in hypospadias and/or a delayed onset of puberty and can be monitored by measuring blood alcohol levels. In addition, the impact of stressful sociological factors may be related to precocious puberty (Cesario & Hughes, 2007). Questionnaire data on socioeconomic status and stress will be collected in the Study.

Chapter 9

Rationale for Exposure Measures

9. RATIONALE FOR EXPOSURE MEASURES

The primary purpose of the National Children's Study exposure measurements is to enable the epidemiological analyses of relations between the priority exposure areas and the priority outcomes, not to provide a comprehensive assessment of all a child's environmental exposures from all pathways. Thus, measures have been selected that reflect the Study's priority areas, can be measured consistently through relevant time periods of the child's life, and are suitable for a large-scale population-based study. Similar to the outcome measures, the collection of the array of exposure measures will allow the examination of exposure-outcome relations to be more inclusive than those specified in the NCS hypotheses. It will also facilitate the investigation of mediating pathways.

Because the NCS seeks to establish relations between environmental exposures broadly defined as chemical, physical, psychosocial, and biological, and various health and developmental outcomes at specific points in a child's life, the exposure assessment for the Study must consider how to measure exposures of varying kinds during the child's different developmental phases. Environmental effects, both adverse and beneficial, could result from exposures either prior to, or concurrent with, the outcome. Further complicating the evaluation of the relations between exposure and outcome is the fact that there may be times in children's development when they are differentially susceptible to the effects of the environmental exposure. Because of the longitudinal nature of the NCS, the Study can examine both the overall effects of exposure and susceptibility within each life stage. Given the multiple hypotheses and their related exposures and outcomes it will be important to measure environmental exposures throughout the child's life as primary exposures in some instances may be key covariates or confounders in others.

Aspects of the NCS that have important implications for the collection of exposure measures include the Study's geographic dispersion and the varied socioeconomic, demographic, and urban vs. rural nature of the study population. These characteristics present challenges to the collection of exposure measures, necessitating consideration of a number of factors, including: the stability of biological and environmental samples; acceptability of data collection processes to various segments of the study population; and, availability of local environmental data or information sources.

The NCS will store many of the collected samples and immediately analyze only those critical to the NCS priority areas and those that are subject to degradation in storage. Many of the environmental samples and biospecimens will be collected, aliquotted, and stored so they can be analyzed later in NCS subpopulations or in nested case-control studies (Sections 9.2, 9.5, and 9.6). This practice maximizes the efficient use of finite samples for future analyses that will be driven by the evolution of research questions, advances in analytic techniques, and availability of funding.

This chapter is subdivided into five major sections that represent different aspects of exposure: demographic, chemical, physical, psychosocial, and biological. Though the characterization of some potential exposures is obvious (e.g., infections as biologic exposures), others are less so (e.g., medication use is listed under chemical exposures). Some broad categories of exposure span two sections (e.g., some aspects of neighborhoods are identified under physical exposures, while other aspects are outlined under psychosocial). The classification of potential exposures here is just one possible organization and cannot reflect the overlapping and extensive nature of the spectrum of exposure assessment measures within the NCS.

9.1 Demographics/Culture

In the context of epidemiologic studies such as the NCS, demographic data refer to individual-level characteristics that can be used to define sub-populations of people within a larger population. Examples of attributes commonly classified under the demographic umbrella are: age; gender; measures or estimates of social position such as education, occupation, and income; and indicators of race, ethnicity, and culture. These data are important to the NCS not only because of the strong association many of them have with the Study's priority outcomes and exposures, but also because of the Study's charge to elucidate causes of existing health disparities among U.S. children.

In the NCS, as in most studies, demographic data will be collected by self-report from the parents with initial proxy reporting for the child. Though the Study's intent is to obtain information pertaining to the biological father and other "father figures" directly from the individual, proxy reporting from the mother will be used if necessary.

Standardized measures of race, ethnicity, education, family income and structure, religion, employment, public program participation, health insurance, financial security, and food sufficiency will be collected on all study participants. Baseline demographic information will be collected from NCS participants beginning in pre-pregnancy for women in the pre-conception cohort, and at the first in-person interview for women who are enrolled after they become pregnant. Key demographic measures will be updated for both cohorts during all face-to-face visits, throughout pregnancy and after birth. Family structure will be established at the first in-person visit by obtaining a roster of household members. The gender, age, race, and ethnicity of each family member, and their relationship to the index female, will be collected. Changes in household composition will be tracked during subsequent face-to-face visits, including visits following the birth of the child. Measures chosen to assess family structure include those used on other large-scale studies (Census 2000; National Health Interview Survey [NHIS]; Survey of Income and Program Participation [SIPP]).

Employment status, educational level, and income will be measured for the mother and father at the initial face-to-face visit using standard questions from SIPP, Census 2000, and the American Community Survey [ACS]. This information will be updated at in-person visits during pregnancy and following the birth of the child. Changes to the mother's employment status will be captured during selected phone contacts during preconception and pregnancy. At the pre-discharge visit, the mother will be asked about her plans to return to work. Information on the parents' religious affiliation and attendance at religious services will be collected at the first pregnancy visit using standard questions from the National Study of Youth and Religion and then not again until after the child's birth.

Information on the mother's and father's country of birth, languages spoken in the home, acculturation, and connection to other cultures will also be measured. During pregnancy and infancy, these factors relate to the behavior and parenting practices of the parents; as the child grows, these factors relate to the ability of the child to acculturate and integrate into the American culture and to perform well in school. Information about cultural practices will also be updated if there are changes in primary caregivers (e.g., new father figure). Changes in these parameters will be charted longitudinally (e.g., the mother marries someone from another culture or ethnicity). Standard items from Census 2000 and the Early Childhood Longitudinal Study-Birth Cohort [ECLS-B] are among the measures used to collect this information.

9.2 Chemical Exposure Measures

Exposure assessment is the process of estimating or measuring the magnitude, frequency, and duration of exposure to an agent, along with the number and characteristics of the population exposed (Needham et al., 2005; Zartarian, Bahadori & McKone, 2005). The primary purpose of exposure assessment in the NCS is to support epidemiological analyses of relations between exposures and outcomes. The exposure assessment framework for chemical agents draws on work of the NCS Exposure to Chemical Agents Working Group (NCS Chemical Agents Workgroup, 2004) and of investigators from the Children's Environmental Health Centers (Kimmel, Collman, Fields, & Eskanazi, 2005). The framework is composed of three concepts:

(1) Core measures will be obtained for the entire cohort, and validation sub-samples will be considered for more intensive exposure measurements. The most precise or detailed chemical measures are often the most intrusive and costly. Targeted sub-sampling efficiently uses those tools, decreases their use in settings in which chemical concentrations would be below detectable limits, and increases the utility of survey-based and indirect measures (e.g., community-based measures applied to individuals) among participants who do not receive those intensive measurements.

(2) A hierarchical approach will be implemented that relates measures obtained at different geographical levels (e.g., individual, residential, neighborhood, and region). Using air pollution as an example, individual-level exposures can be estimated from neighborhood and residential monitoring and other data (e.g., time-activity questionnaires). In some communities, depending on the availability of local ambient monitoring stations and spatial variability of pollutants, the NCS researchers may need to collect neighborhood-level samples for some media (e.g., air, water).

(3) Multiple exposure assessment approaches will be used, including information from environmental and biological samples, questionnaires, diary reports, and physical and visual assessments. In general, biological measurements will be used where biomarkers of exposure are available, especially for persistent chemicals for which there are relatively consistent exposure patterns, and for which knowledge of the route of exposure is not critical. For other chemicals, such as non-persistent pesticides, environmental samples will also be collected at home visits. These results will be combined with questionnaire answers, observations, and neighborhood-level monitoring to estimate total exposure. Where feasible, some environmental samples will be collected prospectively and stored for later analyses to help with the interpretation of biological measurements. Figure 9-1 demonstrates how these assessments come together to yield the "true" level of exposure.

Domains, sub-domains, and example target chemicals were identified in the Study hypotheses either as the primary agents of interest or as potential covariates. Approaches for measuring these chemicals or their metabolites in environmental and biological samples, or for identifying questionnaire- or observation-based surrogates, were identified. Selection of specific methods to estimate exposure depends on the relative importance of environmental and biological measurements, the pathways of exposure, and the timing of related (hypothesized) outcomes by life-stage at each visit/contact. Temporal and spatial variability, along with developmental changes in children's physiology and behavior, were considered in selecting the combination of measures and questionnaire items. For example, in pregnancy, environmental measures with greater temporal stability are combined with short-term biomarkers and questionnaire responses on the frequency of source use to characterize both chronic and intermittent exposures. In contrast, environmental measures and questions are not included when the agent was unlikely to have an effect on the fetus (e.g., potential relation to birth outcome) independent of

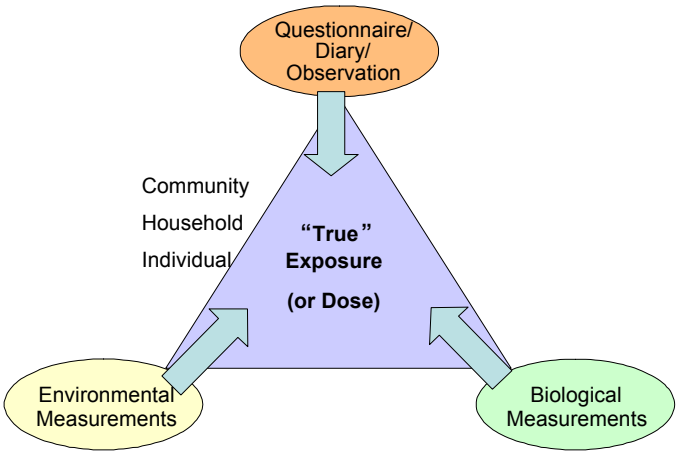


Figure 9-1. True Exposure as a Combination of Multimethod Assessments

that represented by a biomarker taken from the mother. The combined exposure assessment approach is summarized in Table 9-1, with specific measures by contact provided in Appendices F.1, G, and H. Approaches to assessing exposures for the major classes of chemicals are discussed below.

Persistent organic chemicals (POCs) include organochlorine pesticides, polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), polyfluorinated biphenyls (PFBs), perfluorinated chemicals (PFOA/PFOS), brominated flame retardants (PBDEs), and dioxins/furans. Chemicals in this class usually have half-lives of months or years in the environment. POCs are readily absorbed into the blood supply by passive diffusion and distributed into the fatty portions of organs, tissues, and breast milk. During pregnancy, POCs may also distribute in the fetal compartment. Assessment of maternal POC burden, and thus an indirect measure of fetal exposure, can be obtained from maternal blood taken before or during pregnancy and maternal blood, milk, or adipose tissue taken soon after parturition. Fetal and early life exposure to POCs have been hypothesized to be associated with numerous health outcomes, including neurodevelopment and thyroid function, later type 1 diabetes, and reproductive health at puberty.

Because valid biomarkers exist for POCs and are considered the gold standard for measuring persistent compounds (Needham et al., 2005), these will be the primary means of exposure assessment in the NCS. POCs will be measured in the mother's blood and urine in samples taken prior to and during pregnancy. POCs will also be measured in samples of breast milk, cord blood, and the child's blood and urine during early childhood. (See Appendix G for details of samples to be collected by contact.)

The purpose of testing at multiple time points is to determine when the exposure(s) occurred. Because POCs are persistent, it is difficult to determine if elevated levels in biological samples are due to past high-level exposures or recent lower-level exposures, yet this distinction can be important in understanding exposure-dose-outcome relationships. Thus, dust samples will be collected from the homes of all study participants that can be measured later for organochlorine pesticides and other POCs in the subset of mothers/children with high levels in biological samples. Collection of water samples in homes in rural communities, or where community water supplies are reported to have organochlorine

Table 9-1. Summary of NCS Chemical Exposure Assessment Approaches

Approach	Types of samples / Questionnaire domains	Target chemical/agent class (measures) / Topic areas (for questionnaires)
Biomarkers	Blood	PCBs, persistent and non-persistent pesticides, PBDE, perfluorinated compounds, PBDE flame retardant; perchlorate; lead, mercury, cadmium; bisphenol A
	Urine	PFBS, alkyl phenols, Hg(inorganic), As(speciated), perchlorate, halogenated phenols (PCP), phthalates, atrazine, OPs, carbamates, pyrethroids, EBDC/ETU,cadmium
	Breast milk	Dioxins/furans; organochlorine pesticides; PCBs
	Meconium	Cotinine, organophosphate metabolites
	Nails	Mercury (organic, inorganic)
	Hair	Cd, cotinine, mercury, nicotine
Environmental measurements	Indoor Air (Residence, child care locations)	Particulate matter (PM10), NO2, O3, CO VOCs, aldehydes and ketones,
	Outdoor air (community-level)	PM2.5, NO2, NOx, SO2, O3
		Pollen
	House dust	Allergens, endotoxin, mold, metals, pesticides (plus archives for future analyses)
	Potable water	Disinfection byproducts (BBPs), metals, coliforms, nitrate, perchlorate, pesticides
	Soil	Metals, pesticides
	Food	Metals, pesticides
Questionnaire, diary, or observation	Visual assessment	Housing, neighborhood characteristics
	Housing characteristics	Building age, renovations; heating/cooling systems/usage, clothes dryer, vaporizers, air cleaners, stove use, water for drinking and cooking, ozone sources, vacuum cleaner use, garage location and use, gasoline exposure, noise
	Occupational/hobby exposures	Types of jobs, activities, exposures
	Product use	Creams/lotions that are widely applied; cleaning products
	Pets and pesticide use	Type, method, frequency of application, and use protective equipment; number and types of pets, and exposure to flea/tick treatments
	Time and activity	Time spent at home, work/school, in-transit for work and non-work days
	Diet	Food-frequency questionnaire; three-day checklist; infant feeding/intake; eating behaviors (child)
	Related domains/topics	Environmental tobacco smoke, take home exposures, physical activity, household composition and demographics

contamination, will allow assessment of residual levels of organochlorine pesticides in drinking water. Methods will be comparable to those used in the U.S. Department of Housing and Urban Development / U.S. Environmental Protection Agency's First National Environmental Health Survey of Child Care Centers and the National Cancer Institute's New England Study of Environmental Health (NESEH).

Because dietary intake is another important exposure route, information on diet and food preparation will be collected at multiple times throughout the study (before and during pregnancy and during early childhood). Sample food items may also be collected based on those foods identified as being consumed most often. The mother's food frequency questionnaire, three-day checklist, and child's milk and food feeding forms (as used in the National Health and Nutrition Evaluation Survey [NHANES]) will be linked to national environmental contaminant databases, including the U.S. Department of Agriculture's Pesticide Data Program and Food and Drug Administration's Total Diet Study, as well as to a community database of environmental contaminants to be developed for the NCS. The use of flame retardant clothing for the child will also be assessed through questionnaires.

9.2.2 Non-persistent Organic Compounds

Non-persistent volatile organic chemicals (VOCs) include compounds in the air such as formaldehyde, benzene, vinyl chloride, other aldehydes, acrolein, ketones, and disinfection by-products in drinking water such as trihalomethanes (e.g., chloroform) and haloacetic acids.[1] Concentrations of these chemicals vary during short periods of time, depending on use of VOC-emitting products, smoking, ventilation in the home, and treatment and storage of community water supplies. Exposure to VOCs in utero and postnatally is hypothesized to increase risk of asthma, to reduce neurobehavioral and cognitive skills, and to impact the endocrine system and type 1 diabetes.

Although VOCs can be measured in biological samples such as expired air, blood, and urine, VOCs are rapidly metabolized and excreted so measurements made at a specific time will only address the exposures that occurred in the prior few hours. Multiple biological samples taken during pregnancy and childhood can be costly to the Study, burdensome to the participant, and logistically difficult to collect and store. Thus at least initially, biospecimens will not be collected for VOC analysis in the NCS.

Indoor air and drinking water samples will be collected for VOC analyses using methods that have been employed by studies such as The National Human Exposure Assessment Survey (NHEXAS), NHANES, and NESEH. Because of the temporal nature of VOC exposures, week-long average air measurements will be made multiple times during the Study, including pre-pregnancy, during pregnancy, and early childhood. Water collections will be made at homes served by a community water supply during pregnancy and early childhood; samples of the community supply also will be collected.

In addition, questionnaires will address use of VOC-emitting materials and products in the home and elsewhere, occupational and hobby related exposures, traffic exposures, smoking, and home ventilation factors. Observations in and outside the home will also identify sources of VOCs and ventilation.

Non-persistent semi-volatile organic chemicals (SVOCs) include organophosphate and carbamate pesticides, herbicides (including atrazine), polycyclic aromatic hydrocarbons, phthalates, halogenated phenols, alkyl phenols, and environmental tobacco smoke. Like exposure to VOCs, exposure to SVOCs in utero and postnatally is hypothesized to increase the risk of asthma, reduce neurobehavioral

[1] Haloacetic acids are not generally considered to be VOCs, but they are included in this document because it is a class of disinfection by-products of interest, along with trihalomethanes.

and cognitive skills, and impact the thyroid system. Non-persistent nonvolatile organic chemicals (NVOCs) include pyrethroids and other pesticides and phytoestrogens. They are hypothesized to increase the risk of compromised neurobehavioral and cognitive skills, to impact the thyroid system, and to increase risk of type 1 diabetes.

Though SVOCs and NVOCs are generally rapidly metabolized and excreted, there are valid biomarkers for some of these compounds; they must, however, be measured at multiple times. The mother's urine will be collected before and during pregnancy and at birth, the father's urine will be collected during pregnancy, and the child's urine will be collected during early childhood. Organophosphate pesticide metabolites also will be measured in the meconium. Since potentially non-toxic pesticide metabolites may be present in environmental samples, bio-specimens tested for those metabolites may be positive even without exposure to the actual pesticide (Morgan et al., 2005). Thus, environmental samples can be analyzed for both the pesticide and the metabolites to help interpret positive bio-markers.

To assess exposure to environmental tobacco smoke, nicotine and cotinine will be measured in the hair and urine of the mother before, during, and after pregnancy; of the child during infancy; and of the father. The analysis of hair and urine at multiple times will allow for characterization of recent and chronic exposure of the child to environmental tobacco smoke. Interview questions addressing parental tobacco use in the home will be asked at multiple visits, and nicotine may also be measured in house dust during early childhood to help differentiate between inhalation and dermal exposures.

Pesticides will be measured in air, dust, water, and soil before birth and during early childhood, since usage patterns may change during pregnancy and after the birth of a child. To reduce the number of samples that are likely to have non-detectable measurements, air samples for the semi-volatile and non-volatile pesticides will be collected only when a recent application is reported, or in agricultural areas. Dust samples will provide a long-term indicator of potential for exposure. Water and soil samples for pesticides will be collected only in rural areas. Polycyclic aromatic hydrocarbons will be collected in air samples during early childhood.

SVOC questionnaire and observation items will elicit the types and frequency of insecticide use, including flea control products and lice or scabies treatment, smoking, combustion sources, product use, and dietary consumption and sources of foods. The mother's time and activity patterns, including commuting patterns, will be assessed during pregnancy. Questions about the child will include teething and pacifier use, and mouthing of hands and objects which may result in greater exposures to contaminants in dust or on surfaces and toys. Combining these measures with the biologic and environmental samples should allow for identification of both acute and chronic exposures.

9.2.3 Inorganic Chemicals

Bioaccumulative inorganic chemicals include lead, mercury, and cadmium. These metals persist in the environment. Because they are slow to metabolize and excrete, they accumulate in the body as the element itself or as organometallic compounds. Exposure to bioaccumulative inorganic chemicals in utero and postnatally is hypothesized to impact the endocrine system, timing of puberty, neurodevelopment, and type 1 diabetes in the child. Valid biomarkers exist for bioaccumulative metals (Needham et al., 2005) and will be used for exposure assessment in the NCS. Lead, mercury, and cadmium will be measured in the mother's blood before and during pregnancy, cord blood, and in the child's blood during early childhood. Cadmium and inorganic mercury will be measured in the mother's urine before and during pregnancy and in the child's urine during early childhood. In addition, inorganic and organic mercury will be tested in the mother's nails during pregnancy and the child's nails during

early childhood. Cadmium and organic and inorganic mercury will be measured in the mother's hair pre-pregnancy and during pregnancy, the father's hair during pregnancy, and the child's hair during early childhood. Hair and nails account for a longer term exposure and are easy and cost effective to obtain and to store. These specimens will be collected at visits throughout childhood.

As with the persistent organic compounds, relevant environmental samples will also be collected and stored so that those with high biomarker concentrations (and a sample of others) can be analyzed to help determine whether the high concentrations are due to current exposures. Lead and cadmium will be measured in air and soil samples several times before, during, and after pregnancy. Lead also will be measured in house dust and drinking water during pregnancy and during early childhood. Methods will be comparable to those used in NHEXAS, the National Survey of Lead and Allergens in Homes, and NESEH. Mercury measures in the environment are not included as core Study measures; however, this could be done as an adjunct study in areas where this is of concern.

Non-bioaccumulative inorganic chemicals include arsenic, chromium, manganese, nitrate, and perchlorate and can be measured in air, dust, water, food, and soil. These chemicals are readily absorbed into the body; some distribute to various tissues and others are rapidly excreted. Exposure to non-biaccumulative inorganic chemicals in utero and postnatally is hypothesized to impact the child's thyroid system, neurodevelopment, and type 1 diabetes. In general, measurements of these chemicals in hair will offer a longer-term dosimeter for exposure, while urine will provide an assessment of more recent exposure.

Perchlorate, manganese, and other metals will be measured in the mother's breast milk. Arsenic (speciated) and perchlorate will be measured in the mother's urine before and during pregnancy and at birth, in the father's urine during pregnancy, and in the child's urine during early childhood.

Manganese will be measured in air samples taken at multiple times during the Study, since inhalation may be the most toxic route of exposure. Arsenic will be measured in house dust and drinking water during pregnancy and during early childhood, in soil samples around the house and near CCA-treated wood during early childhood. Nitrate and perchlorate will be measured in drinking water only in rural areas.

Questionnaires and observations will focus on the home's age, renovations, and source of drinking water. Observations and geographic information system (GIS) data will also identify nearby industrial sources. Occupational and hobby questions will highlight metals exposure at work or brought home, as well as other occupation-related exposures. Dietary ingestion is likely to be an important exposure route for most metals. Thus, as for persistent organic chemicals, dietary consumption information will be collected before and during pregnancy and during lactation for the mother, and after birth for the child. Food preparation information during pregnancy also will be collected.

9.2.4 Criteria Air Pollutants

Criteria air pollutants include particulate matter (PM), carbon monoxide (CO), nitrogen oxides, and ozone. Air pollutant exposure depends on ambient concentrations, season, traffic patterns, and indoor combustion and ventilation. Biomonitoring currently has a limited role in the assessment of exposure to criteria pollutants. While CO can be measured in blood or expired air, this is burdensome and reflects only short term exposures. Exposure to air pollutants during pregnancy, infancy, and childhood is hypothesized to increase the risk of asthma and wheezing.

Active and passive sampling and measurement using direct reading instruments are the most common form of environmental assessment tools for these air pollutants. In general, biomonitoring has a limited role. While personal sampling better reflects an individual's exposure, the logistical and burden demands of personal sampling have led the NCS to adopt indoor sampling in rooms or areas where the participants spend the most time. Particulate matter (<10 micron) samples will be collected before, during, and after pregnancy to assess the variability in exposures over time and season. Nitrogen oxides and CO will be measured during pregnancy and during early childhood. Ozone will be measured starting in early childhood and only in homes with ozone sources present. Methods will be comparable to those used in NHEXAS.

PM measurement and some gaseous air pollutants may also occur at the community level, where existing monitoring sites fail to provide coverage of the selected communities or where spatial variability is high due to local sources. Adjunct studies may also explore person-level exposures for various pollutants, e.g., traffic-related exposures, to help evaluate and adjust for measurement error in assessments based on measures of locations by accounting for individual differences in proportions of time.

Questionnaire items and home observations will focus on heating and cooking fuels and use patterns, exhaust ventilation of the home, and ozone sources. Additionally, observational and GIS data will identify sources of air pollutants in the neighborhood. Activity patterns on working and non-working days will be collected, including time spent in different locations and information on in-vehicle exposures.

9.3 Physical Exposure Measures

As children mature their physical environment, and thus many environmental exposures, expand from being primarily the home to a broader array of locations, including childcare settings, schools, neighborhoods, and recreational facilities. The NCS will collect data describing the physical exposures at a number of these locations through the child's life. Resource and participant burden considerations will limit data collection activities at each of these locations. Since even up to age 5 the average child spends the majority of time indoors at home [up to 16 hours daily (Hubal et al., 2000)], the child's home environment(s) receives the greatest consideration early in the Study.

The NCS will adopt many of the basic questionnaire items and observations from already established surveys, such as the American Housing Survey, but in the interest of participant burden, it will limit questions to factors related to chemical and biological exposures in the environment, injuries to children, opportunities for recreation and physical activity, and access to services such as health care and shopping. A summary of assessment approaches for the physical environment exposures appears in Table 9-2 below, and detailed information appears in Appendix H.

9.3.1 Housing Characteristics and Condition

Housing characteristics generally describe the physical configuration and condition of the residential structure. The physical configuration includes such information as the date of construction; materials used for the initial construction and subsequent renovations; the heating, ventilation, and air conditioning systems; the electrical and plumbing systems; floor covering; the presence of major appliances such as stoves and fireplaces; and aspects of the land on which the residential structure is built. Housing condition refers to the existing state of maintenance of the house, including structural integrity, presence of functioning electrical supply, integrity of painted surfaces, and the coverage of the site by

grass, dirt, or other material. Additional housing-related physical exposures include indoor temperature and humidity, and noise.

Table 9-2. Summary of NCS Physical Exposure Assessment Approaches

Approach	Domains	Topics within domain
Questionnaire or observation	Housing characteristics and condition	HVAC, structural integrity, integrity of painted surfaces, insects/rodents, floor covering, presence of major appliances, fireplaces and exhausts
	Visual assessment of neighborhood	Type and condition of housing, presence and type of businesses, industries, recreational areas, graffiti, safety, traffic
Maps and databases	Geographic information systems data	Superfund and brownfield sites, Superfund Amendments and Reauthorization Act (SARA) reporting sites, nearest hospitals/medical care, recreational facilities, traffic
	Aerial photography	Geographical details (rivers, mountains, highways)
	Census data	Population density

Household characteristics and condition will be assessed through a combination of participant questionnaires and direct observations by Study personnel (see Appendices F.1 and H). To minimize burden, questions for the participant will be limited to those that cannot be easily observed, e.g., date of construction and use of special filters on the ventilation system. Most housing characteristics will be recorded via direct observation. Observation forms will parallel those used in many other studies, including HUD/NIEHS National Survey of Lead and Allergens in Housing (Vojta et al., 2002), HUD/EPA's First National Environmental Health Survey of Child Care Centers (Marker, Fraser, & Viet, 2001), HUD/EPA's American Healthy Homes Survey, and the Cincinnati Children's Environmental Health Center–HOME Study.

Most housing characteristics and conditions are relatively stable in the short-term and therefore require only periodic updating. In consideration of participant and study burden, assessments will focus on the characteristics and conditions most relevant to the current life stage of the Study children. For example, recording condition of paint on walls and sills, or recent home renovations, will occur throughout the Study since potential lead or other exposures are germane during pregnancy as well as childhood. However, home safety assessments potentially related to childhood injury (e.g., burns, falls, poisoning, drowning) will be initiated after the birth of the child.

In addition to the physical assessment of the child's home, the NCS plans to collect similar data from sites of child care and, as the Study progresses, schools. The extent to which these assessments will be obtained through direct observation by Study personnel, through self-assessment by the child care site, or through other means, has not yet been determined and will be influenced by a number of factors including cost and ability to gain access to the various child care sites.

9.3.2 Neighborhood Characteristics

Neighborhood characteristics extend the description of the child's environment beyond the boundaries of the structure in which they live. The influence of a child's neighborhood environment on his or her development may arise from both physical characteristics (e.g., amenability to outdoor activity or proximity to a hazardous waste site) and social factors (e.g., community cohesion and collective efficacy). Although the physical and social aspects of a neighborhood are related, this section

concentrates on the assessment of the neighborhoods' physical characteristics and leaves the description of social factors to Section 9.4.

Features of the physical or built environment of interest to the NCS include conditions that influence physical activity, safety, access to nutritious foods, and exposure to chemicals. Numerous aspects within each of these four features will be measured to ensure comparability of findings from the NCS to previous studies that examined health effects related to the physical or built environment. Aspects of these four features frequently overlap or are inter-related. These aspects include, but are not limited to: population density; residential density; neighborhood vegetation or green space; land use mix; safe walking/cycling locations; high speed traffic; heavy traffic; proximity to intersections of major highways or railroads; intersection density (connectivity); lack of crosswalks and sidewalks; access to trails; density of bus and subway stops; urban sprawl; dependence on motorized transportation; and street connectivity.

The physical or built environment is of increasing interest primarily because of its linkages with physical activity and obesity. Residential and population density promote mass transit usage and walking as a means of transportation (Frank, Andresen, & Schmid, 2004; Frank, Engelke, & Schmid, 2003; Ross & Dunning, 1997; Saelens, Sallis, & Frank, 2003). Street connectivity, or the density of intersections, affects the ease with which individuals can walk in a direct path to their destinations (Frank et al., 2003). Mixed land use is a good predictor of walking because areas with a higher mix of commercial and nonresidential destinations facilitate walking as a means of accomplishing daily activities, which reduces the risk of obesity (Frank & Pio, 1995; Handy, 1996; Sallis, Saelens, & Kraft, 2004). Access to leisure facilities including public gymnasiums, swimming pools, and soccer fields is related to recreational walking, but not to walking as a means of transportation (McCormack, Giles-Corti, & Bulsara, 2007). Access to commercial food sources are consistently related to walking for transportation, however, the type of store is equally notable as convenience stores often stock unhealthy foods (Sallis, 2007). Urban sprawl, as measured by a number of different indices, has been consistently associated with risk of obesity among U.S. adults (Ewing, Schmid, Killingsworth, Zlot, & Raudenbush, 2003; Lopez, 2004) and adolescents (Ewing, Brownson, & Berrigan, 2006). The underlying mechanism of urban sprawl's effects (decreased population density, low street connectivity) may originate from greater dependency on motorized transportation, decreased ability to walk to destinations, and environmental degradation such as more greenhouse gas emissions and reduction of open spaces that facilitate walking and physical activity (Lopez, 2004).

Different aspects of the built environment may interact with or confound each other when predicting likelihood of physical activity and obesity. Low urban sprawl (more sidewalks, greater population density) may facilitate walking for exercise, but may be associated with higher crime rates, which deters walking. Therefore, the inter-relations among aspects of the built environment are complex and require the assessment of multiple characteristics of the built environment.

The built environment has also been linked with outcomes other than obesity. Children living in census tracts that faced intersections with highways or railroads had a 60 percent increased risk of developing asthma compared to children who did not live in census tracts facing intersections, after adjustment for individual- and neighborhood-level covariates (Juhn et al., 2005). Neighborhood physical environment characteristics may be associated with chemical exposures, including the presence of industrial facilities such as incinerators, recycling facilities, chemical manufacturing or mining operations, and hazardous waste sites.

The methods by which aspects of the built environment will be obtained in the NCS include parental report interview, direct observation by Study personnel, and the examination of existing maps and databases. In the NCS, the majority of objective information concerning the neighborhood physical environment will be obtained via direct observation using standardized tools. Items to be recorded as

observed from the dwelling structure are presented in Appendices E and H. Though standardized and relatively objective assessment of the built environment is a comparatively recent development, numerous instruments have been developed (e.g., SPACES, Irvine-Minnesota Inventory). These and other similar instruments will be considered for use on the NCS. The selected instrument must be well-validated in several different settings and must capture a range of built environment characteristics.

To obtain information that cannot be observed from the home, Geographic Information Systems (GIS) may be used to identify industrial areas and facilities in the neighborhood and may be integrated with aerial photography (where available) and local census data to measure aspects of the built environment objectively such as intersection density, street connectivity, population density, residential density, and land use mix. This secondary type of community-level data will be linked to the NCS data.

In addition to observational and GIS data, the NCS will also collect respondents' perceptions of their neighborhood via questionnaires. Although objective measures obtained by GIS and by measures of individuals' perceptions of the built environment have poor agreement, each is independently associated with physical activity (McGinn et al., 2007). Thus, it is important to address both objective and perceived measures of the built environment in the NCS.

9.4 Psychosocial Exposure Measures

Psychosocial and behavioral factors have broad-reaching effects on children's health and well-being, and are linked to key Study outcomes. The identification of psychosocial domains to be included in the NCS is based on the exposures and covariates named in the core hypotheses, on developmental white papers commissioned by the Study, and on workshops held on specific topics (e.g., parenting, racism/discrimination, media exposure, prenatal stress, gene-environment interactions). The measures have been selected or adapted from established, well-validated, standardized instruments utilized in other (primarily epidemiologic) studies.

An important requirement for the selected measures is that they have the ability to measure the same construct (e.g., parenting practices) reliably through time and to capture changes in exposures to the individual child through different developmental stages. They should also have the ability to track societal changes (e.g., changes in racism/discrimination) or trends across time. The most reliable way to document these influences on child development is to measure the same construct at each participant contact. Given the breadth of exposure domains in this large, complex study, and the broad scope of psychosocial factors (family influences, child care, media, neighborhoods, socioeconomic status, school environment, etc.), repeating all domains at every visit would create interviews that far exceed a reasonable participant burden. The schedule for administration of psychosocial measures has therefore been designed to provide data primarily at critical time points for each domain. Decisions were based on careful deliberations and the advice of many outside experts. Criteria used in the evaluations of measures included sound psychometric properties, logistical feasibility (time, burden) for testing in the home, and flexibility of administration modalities to minimize respondent burden and cost. Other criteria used for selection of measures were that they be relatively easy to administer and score, be unbiased (e.g., against low-income, minority or cultural groups), and be sensitive to individual variations. An important consideration also included suitability for translation into multiple languages.

As with the chemical exposures, these measures also involve hierarchical data. There will be community level data (e.g., crime levels, percent of inhabitants on welfare), neighborhood effects (e.g., collective efficacy), school characteristics, and individual exposures. These factors will interact with each other in creating a child's psychosocial environment and can be dealt with analytically through hierarchical analyses (see the Statistical Analysis Plan, Chapter 10). A summary of assessment

approaches for the psychosocial exposures appears below in Table 9-3; a list of questionnaire, interview, and direct assessment measures at each visit up to age 2 can be found in Appendix F. Most of the psychosocial domain will involve interviews with relevant respondents, observations in the home, and in some cases, verification with biological specimens (e.g., catecholamines and cortisol for stress).

Table 9-3. Summary of NCS Psychosocial Exposure Assessment Approaches

Approach	Domains	Topic areas
Questionnaire or observation	Demographics and culture	Household composition, age, ethnicity, country of origin, languages spoken in the home, income, education, religious affiliation, employment, resources
	Family process/environment	Family structure, parenting, dyadic relationships, home environment, domestic violence
	Maternal depression	Prenatal, postnatal depression
	Psychosocial stress	Prenatal life events, perceived chronic stress, racism/discrimination
	Social support	Emotional support, instrumental support, network support
	Neighborhood and community	Collective efficacy, social cohesion
	Health behaviors	Smoking, alcohol consumption, physical activity, substance abuse
	Child care and schools	Structural and qualitative aspects
Biomarkers	Saliva	Cortisol (diurnal variation)
	Urine	Cotinine
Extant databases	Neighborhood and community	Examples reviewed for possible use: Subsidized households; Neighborhood Change Database; School District Data Book; Uniform Crime Reports; Office for Civil Rights Census of Schools; Census of Agriculture; County Business Patterns; occupational employment statistics; American Housing Survey Area Resource File; Behavioral Risk Factor Surveillance Survey; Bureau of Economic Analysis: data on per capita income; Gardiner Tobacco Data Health Care Finance Administration File; NCHS Compressed Mortality File; NCHS Vital Statistics Data and Death Index; state and local employment and unemployment rates; State and Metropolitan Area Data Book; etc.

9.4.1 Family Process/Environment

Family environment has a consistent and enduring influence on a child's social, emotional, and cognitive development. The security of attachment the child has with parents or primary caregivers influences their relationships with teachers and friends (Shonkoff & Phillips, 2000), memory processes (Belsky, Spritz, & Crnic, 1996; Kirsh & Cassidy, 1997), self-concept (Verschueren, Marcoen, & Schoefs, 1996), and conscience development (Kochanska, 1995, 1997). Parenting practices and home environments are also an important source of cognitive stimulation for literacy and numeracy skills, as

well as for language development (Snow, 1993; Ginsburg, Klein, & Starkey, 1998; Bradley et al., 1989). In addition, the family environment strongly influences a child's learning of self–regulation (Cummings & Davies, 1994) and conflict resolution (Thompson, 1988). Parents act as managers of their children's environment and influence them through multiple pathways, including parent-child interactions, parenting knowledge and attitudes, cognitive stimulation, and stress modulation.

The family environment as it is defined in the NCS includes household structure, the quality of relationships among household and family members, media use, domestic violence, division of labor, parenting behaviors, and parental mental health and cognition. Due to flexible family configurations tied to divorce, remarriage, and non-married cohabitation, the family environment requires re-assessment at regular intervals. Parenting ability can also vary at different developmental stages and should be measured longitudinally. The measures described below are those that have been selected for pregnancy and early infancy. For time periods when each is measured see Appendix F.1. These constructs will be measured throughout childhood and adolescence, although the specific instruments may vary according to developmental stage.

Family environment will be measured in early childhood with the Infant/Toddler Home Observation for Measurement of the Environment (IT-HOME), an observational instrument designed to describe the attributes in a young child's environment that contribute to social and cognitive development. It is designed for infancy from birth to age 3 and has six subscales: parental responsivity, acceptance of child, organization of the environment, learning materials, parental involvement, and variety in experience. It has been used extensively in multiple longitudinal studies (National Institute of Child Health and Human Development [NICHD] Study of Early Childcare; National Survey of Child and Adolescent Well-being; National Longitudinal Survey of Youth). The HOME can be used at multiple stages of child development.

The quality of the marital or partner relationship impacts parenting competence, and interacts with parental mental health and domestic violence in predicting child outcomes. The Dyadic Adjustment Scale (DAS-7) will be used to assess the quality of the parental relationship. This short version scale, validated by Hunsley, Best, Lefebvre, and Vito (2001), provides three relationship subscales: dyadic consensus, dyadic cohesion, and general satisfaction. It will be administered early in pregnancy and periodically throughout childhood.

Domestic violence will be measured with the Modified of Abuse Assessment Screen (AAS), a clinical instrument that measures frequency and severity of abuse of women. Test-retest reliability is high, and it has been validated in ethnically and socioeconomically diverse samples. This measure will be administered to the mother early in pregnancy and at later stages of her child's life in a way that allows her to respond accurately even if the husband is present in the room.

Division of child care responsibilities within the family will be assessed using the "My Time as a Parent" measure, which has been validated in major longitudinal studies (NICHD Study of Early Childcare). This will be measured after the child is born, and at age 6 months as the duties of both parents increase.

Parenting practices and behaviors will be assessed in both mothers and fathers throughout childhood. In early childhood, specific questions will be similar to those used in the Early Childhood Longitudinal Study–Birth Cohort (ECLS-B). Domains will include wantedness of the child, parenting activities and practices involving decisions, looking after the child, meals, and attitudes about being a parent. Observations of standardized parent-child interactions will also be videotaped for later coding. The Three Boxes Task is a semi-structured activity completed by the parent and child in interaction. Parental sensitivity, parental intrusiveness, cognitive stimulation, parental positive regard, parental

negative regard, and parental detachment are assessed. Three scales assess child behavior: engagement with the parent, sustained attention, and negativity toward the parent. The Three Boxes Task has one of the few coding systems that can be used in large-scale studies, has good psychometric properties, and produces robust scores predictive of later development in both cognitive and socioemotional domains. It has been validated in several large-scale studies (NICHD Study of Early Childcare; Early Childhood Longitudinal Study–Birth Cohort [ECLS-B]; Early Head Start Research and Evaluation project).

Media use will be assessed by questions to the mothers throughout childhood beginning when the child is six months old. Topics will cover the amount of time the television or radio is on; how often the child watches television or movies, plays video games, or listens to music; the extent of exposure to books and other reading material; and the content of the media exposure.

Parental mental health and cognition will be measured in both mothers and fathers. Domains include intelligence, literacy, depression, and anxiety. Parental depression, which influences not only parenting behavior but is also a potent stressor during pregnancy (Lundy et al., 1999) will be measured using the Center for Epidemiological Studies–Depression scale (CES-D)(Radloff, 1977) during and after pregnancy. Anxiety, also an important influence on parenting, will be assessed with the Spielberger State-Trait Anxiety Inventory (STAI) scale.

To measure potential genetic and cognitive influences of parental IQ on the child, the Kaufman Brief Intelligence Test, Second Edition (KBIT-2) will be used. It has the advantage of including a non-verbal scale (in addition to a verbal one) which is relatively invulnerable to SES factors and language background. It yields scores similar to other intelligence tests, with a mean of 100 and a standard deviation of 15, making it possible to compare KBIT-2 scores with other measures of IQ. The Woodcock-Johnson-III Tests of Achievement Letter-Word Recognition subtest, which measures the individual's word decoding skills, will also be administered.

9.4.2 Psychosocial Stress and Social Support

Psychological stress is the distress experienced by individuals who feel overwhelmed and unable to cope with the demands in their lives. These demands may arise from varying or multiple sources (work, partner relations, family responsibilities, financial insecurity, social isolation, neighborhood issues, racism, etc.) but the emotional experience of distress is the mediator of the detrimental physiological and behavioral responses that occur. It is important to understand that the outside demands that may be a source of distress for one person will not necessarily be stressful for another. It is the experience of distress that starts the cascade of physiological reactions known as the "stress response." For these reasons, we will measure both the participants' global experience of stress, and the sources that may be affecting it. To qualify for the NCS, measurement of these exposures must be germane to pregnancy and/or parenting, or, in the child, to developmental outcomes. They must have high validity across varying ethnic groups, socio-economic levels, urban/rural settings, and religions; and include good psychometric properties and low subject burden. Interview questions will be taken from already validated measures and biological measures of stress hormones (cortisol in saliva). To understand why one person tolerates more demand than another, factors that can serve as buffers against stress (e.g., social support) will also be assessed. Due to the changing nature of stressful situations and the increase in effect when they become chronic, these measures will be administered to parents and children at repeated time points throughout the Study, starting in pregnancy.

Global perceived stress will be measured with Cohen's Perceived Stress Scale (PSS) during pregnancy and after birth. The questions are general and are relatively free of content specific to any sub-population group.

Racism/discrimination, a possible source of stress in some populations, will be measured in the mother during pregnancy and in early childhood with the Experiences of Discrimination (EOD) questionnaire, modified for the Coronary Artery Risk Development in Young Adults (CARDIA) Study. This questionnaire was chosen because it has been validated in other national studies (e.g., the National Study of Youth and Religion) and because it also allows for the measurement of discrimination based on sexual orientation or disabilities. As the child develops, measures of discrimination will include other (e.g., school) environments relevant to the child. While empirical studies of discrimination have been done on African Americans, little research has been done to address systematically how prejudice and discrimination affect other racial/ethnic minority groups (Cain & Kington, 2003). Given the existing health disparities in this country, a measure of discrimination will be pertinent not only as an independent predictor of family influences on child development, but also as a covariate in other hypotheses related to socioeconomic status.

Prenatal life events refer to stressful life events that have happened to the respondent or a spouse or a partner since the respondent became pregnant. Prenatal life events have shown associations with birth weight and birth outcomes (Lobel, 1994). The Prenatal Life Events Scale (PLES) was developed for use in pregnancy and adapted from Epidemiological Catchment Area studies.

Parenting stress will be measured in early childhood with a validated short form of Abidin's Parenting Stress Index (PSI) (from the NICHD Study of Early Child Care). This instrument is designed to identify parent-child systems that are under stress and at risk for development of dysfunctional parenting. The PSI has good psychometric properties and is appropriate for use with parents of infants.

Family/work stress will be assessed with the Work and Family Conflict Scale, which measures strains associated with combining work and family. This scale has been validated in other large scale studies (NICHD Study of Early Childcare). It will be administered in early childhood and periodically throughout childhood.

Financial stress questions have been adapted from several large studies (Fragile Families; ECLS-B; U.S. Department of Agriculture Food Security Scale) and include owning a home, having a bank account, being able to pay the monthly bills, and having food security. These questions will be administered several times throughout the Study.

Social support will be assessed with Sarason's Social Support Questionnaire (SSQ) Short-Form. An extensive program of research using the SSQ, both Long-Form and Short-Form, shows the SSQ to be valid and highly internally consistent. The SSQ is a quantitative and qualitative measure of social support. Social support in the mother will be measured early and late in pregnancy and again during early childhood. Later, it will be measured in the child.

9.4.3 Neighborhood and Community

This section outlines the methods to be used in the assessment of a child's neighborhood's social characteristics (e.g., social cohesion, collective efficacy, safety, social capital, crime statistics, and average SES level) that may influence health and development. Assessment of the neighborhood's physical attributes was described in section 9.3.2.

Multiple sources will be utilized for neighborhood measures in the NCS. Extant databases can be used to provide data such as crime statistics, unemployment rates, average income and education, and disparity measures. The participant's subjective evaluation of relevant neighborhood characteristics will be assessed by interview. A specific measure that may be used is an adaptation of the Neighborhood

Environment for Children Rating Scales, used in the Project on Human Development in Chicago Neighborhoods (Coulton, Korbin, & Su, 1996). Examples of the neighborhood attributes described by this tool include the participant's evaluation of social capital (e.g., community organizations), collective efficacy, extent of institutions and social services (Coleman, 1988; Sampson, Raudenbush, & Earls, 1997).

9.4.4 Child Care/Schools

For children receiving non-parental child care, the potential influence of that care on their development may be through one, or both, of two broad areas. The structural aspects of child care outside the home and schools include the amount of time a child spends in care outside his or her home; whether it is home-based or center-based care; the training and experience of the child care providers; the ratio of children to caregivers; and the age ranges of the other children. Alternatively, qualitative aspects of the care received by the child may include activities providing cognitive stimulation; discipline techniques; and stressors (e.g., noise, bullying, violence, racism/discrimination) inherent in the child care or school environments. Normal developmental progress of the child, changes in child care arrangements, and school advancement necessitate repeated measures as structural and qualitative aspects of care change as the child moves from context to context over the course of development. Structural aspects of a child's early child care experience can be collected through a variety of methods, including parental report, reports from care providers at the facility, or through direct observation of the environment by Study personnel. Qualitative aspects can also be assessed through those modalities; however, the "gold standard" for assessing qualitative aspects is by direct observation using a structured instrument.

In the NCS, both structural and qualitative aspects of child care will be ascertained through maternal report. A number of large studies in the U.S. have collected information about structural and qualitative aspects of child care, including the National Child Care Survey, the National Household Education Survey, the Early Childhood Longitudinal Study-Birth Cohort, and National Longitudinal Survey of Youth. The NCS will use similar instruments to those used in these large studies.

As described in Section 9.3.1, the NCS plans to collect direct observations from at least a sub-sample of participants' child care settings. The Study of Early Child Care and Youth Development, and the Early Childhood Longitudinal Study – Birth Cohort, both used direct observation to collect a combination of structural and qualitative data. A tool will be adopted from those instruments used in these studies.

9.5 Biological Exposure Measures

A child's biologic environment covers a swath of potential exposures, from in-utero interaction with maternal physiology (e.g., maternal glucose metabolism, thyroid hormone levels, or response to infection) to direct contact (primarily, but not solely, after birth) with allergens or infectious agents. As will be the case with all NCS data collection, a balance must be struck between relying on biologic samples and tests considered "diagnostic" or "gold standard," particularly in a medical or clinical research setting, and those appropriate for use in a large, diverse, population-based epidemiologic study. Discussion of maternal glucose metabolism assessment in relation to the potential association with serious structural birth defects (Section 9.5.3) elucidates some of the trade-offs faced in collection of biologic exposures. In addition to the biospecimens, information relevant to biologic exposures will be collected via other modalities. For example, history of recent infectious disease can be obtained through a questionnaire or a health diary.

The broad implications of an individual's genetic characteristics, and their interaction with environmental exposures, including chemical, psychosocial, and biologic, are discussed below. A summary of the assessment approaches for biological exposures appears in Table 9-4, and in detail in Appendix I.

Table 9-4. Summary of NCS Biological Exposure Assessment Approaches

Approach	Types of samples / Questionnaire domains	Target analytes (measures) / Topic areas (for questionnaires)
Biomarkers	Blood (maternal, child, or cord)	Cytokines and chemokines, immunoglobulins, Hgb A1c, fasting glucose and insulin, lipids, adipokines, thyroid studies, corticosteroid studies, estrogens, progesterone, dietary antioxidants, folate, CBC, lymphocyte subsets, DNA, RNA
	Urine (maternal)	Infection (PCR)
	Breast milk	Cytokines and chemokines, immunoglobulins, macro and micro nutritional components
	Placenta, umbilical cord	Histology for inflammation and infection, cytokines and chemokines, immunoglobulins, DNA
	Saliva (maternal)	Cortisol, periodontitis-specific IgA
	Vaginal swabs	Gram stain, cytokines and chemokines, metalloproteinases
Environmental measurements	House dust	Endotoxin, pollens, molds, other allergens
Questionnaire, diary, or observation	Housing characteristics	Mold, pet-related and other allergens
	Health behavior and status (maternal or child)	Recent illness or fever, chronic conditions, mental health, dental health, reproductive history, health care use, stress, sleep, physical activity, diet and nutrition, medication and supplement use
	Family medical history	Child's parents, siblings, grandparents, aunts, uncles

9.5.1 Allergens

The development of asthma in particular, and atopy in general, may be strongly influenced by early-life antigenic exposure. The appropriate development of antigen-specific immune response, and the general evolution to a mature TH-1 inflammatory response, is likely influenced by the interplay between timing of initial infection with viral or other infectious agents and contact with microbial or other antigens. Differences in the timing of initial exposure to allergens, the nature of the specific antigenic exposure, and whether the exposure was preceded by a viral or other infection may help explain contradictory findings suggesting that early infections can be both protective for asthma (hygiene hypothesis) and associated with an increased risk of asthma. Elucidating the contributions of allergic exposure, infection, and inflammation to asthma and other inflammatory-related conditions will be an important challenge for the NCS.

Common allergens of interest to the NCS include cat, dog, mouse, rat, cockroach and mite antigens, and multiple varieties of pollen and molds. In addition, the TH-1 inducing effects of lipopolysaccharide endotoxin suggests that, when not associated with sepsis or an overt bacterial infection, the health-related effects of endotoxin exposure are more closely related to "allergic" response than to any potential infection from the endotoxin-producing organisms.

Biomarkers for allergen exposures include specific immunoglobulin measures in the mother during pregnancy and at birth and in the cord blood at birth. Because the child's blood may not demonstrate specific antigenic response in infancy, and because blood will not be drawn from the child until age 1, environmental samples are important for assessing early allergen exposure. Many of the above allergens, including endotoxins, will be measured in household dust samples. Methods will be comparable to those used in HUD's National Survey of Lead and Allergens in Homes, HUD/EPA's First National Environmental Health Survey of Child Care Centers, and NHANES. Assessment of pollen exposure will rely on established monitoring data in conjunction with regional pollen studies, where supplemental data is needed.

A panel of 36 mold species can be measured in the mold dust samples using a mold-specific Quantitative Polymerase Chain Reaction (QPCR) method developed and licensed by the EPA. This method was selected in lieu of traditional culture methods because of its quantitative nature and simplicity of sample collection. Only 5 mg of sieved vacuum dust is required for analysis, and samples can easily be stored and analyzed later.

Relevant questionnaire items will focus on recent home renovations, activities used to control allergens in the home, the infant's bedding and sleeping environment, and the presence of dogs and cats in the home. Household observations will include assessment for mold sources both in and outside the home.

9.5.2 Infections and Inflammatory Mediators

Maternal or early childhood exposure to different microorganisms (manifest bacteria, viruses, and fungi) has tentatively been implicated in the development of several health outcomes of interest to the NCS, including neurodevelopment and psychiatric disorders, asthma, and type 1 diabetes mellitus. In addition, links between maternal genital tract infection and preterm birth are well-recognized. In contrast to the immediate and direct suppurative effects of infection, such as the cognitive and hearing loss associated with bacterial meningitis, some of the association between infection and the above outcomes is thought to be due to the distal influence of host inflammatory mediators produced in response to infection, as well as to the influence of infection on the maturation of a child's developing immune system.

Identification of infection and inflammation in a medical or a clinical research setting generally involves microbiologic or biochemical analysis of biospecimens. Current or recent infection or colonization with specific organisms can be identified through culture, through molecular fingerprinting (e.g., PCR DNA amplification), or through direct visualization (e.g., Gram stain and microscopy). Host immunoglobulin response to specific organisms can identify recent or historic infection. Non-specific inflammatory response is diagnosed using combinations of up and down regulating cytokines as well as other non-specific markers such as C-reactive protein (CRP).

The NCS will obtain multiple biospecimens from the mother and the child, at multiple times, to enable assessment of infectious and inflammatory exposures. Specimens will be collected, processed, and stored in such a manner that all the laboratory modalities listed in the paragraph above will be possible, with the exception of culture. The logistic difficulties of assuring standardized handling of multiple samples in a field setting and the variety of culture media and techniques needed to grow the plethora of organisms of potential interest make culture untenable for the NCS. Examples of biospecimens to be obtained include maternal blood and urine (once before pregnancy from women in the pre-pregnancy cohort and at several times during pregnancy for all women); vaginal swabs during

pregnancy for Gram stain and for cytokine identification; cord blood and neonatal heel stick blood; breast milk to enable analysis of immunoglobulins or other maternal factors transferred directly to the child after birth.

In addition to the biospecimens, other modalities will be used to collect indirect data relevant to infectious and inflammatory processes. Questionnaires will assess history of infection and fever in the mother and child. Information collected on family composition, particularly siblings, and on child care and school attendance can be used as proxies for viral exposures. The health care visit log will enable tracking of physician visits related to infections. Dust samples collected from the home prior to birth and during infancy provide estimates of early life exposure to endotoxins.

9.5.3 Maternal Glucose and Glucose Metabolism

An increased rate of structural birth defects among children born to women with type 1 diabetes mellitus is generally interpreted as demonstrating the teratogenic effect of fetal exposure to high levels of glucose. Studies also suggest, though not with unanimity, an association between fetal exposure to maternal diabetes and later obesity or insulin resistance. Fetal response to high maternally-derived glucose load, transient increases in in-utero insulin production, and subsequent permanent changes ("programming") in fetal and child metabolism are the presumed factors driving this association. Most research examining fetal exposure to elevated maternal glucose levels examined populations of women with pre-existing diabetes or gestational diabetes. It is interesting that among pregnant women without pre-existing diabetes, glucose metabolism appears to become more efficient in early pregnancy before deteriorating as pregnancy progresses. The challenge facing the NCS is to examine whether sub-clinical impaired glucose metabolism, perhaps even with clinically normal maternal glucose levels, is associated with adverse child health.

Using criteria promulgated by the American Diabetes Association, clinical diagnoses of diabetes and of "impaired glucose tolerance" are made using a combination of fasting glucose levels, casual glucose levels, or the results of a two-hour oral glucose tolerance test (OGTT). Gestational glucose intolerance or diabetes is commonly assessed at approximately 24 weeks gestation using an oral glucose tolerance screen, with follow-up as indicated. Though powerful as diagnostic tools, the above measures cannot assess subtle alterations in glucose and insulin metabolism which might result in normal serum glucose levels. The "gold standard" assessment of insulin resistance used in targeted clinical studies, the euglycemic clamp, is clearly not suitable for the NCS due to issues of participant burden. The ability to obtain fasting serum insulin and glucose levels early in pregnancy is also questionable because the initial NCS contacts will occur in the home environment. It will be difficult to schedule visits around an eight hour fast, and the capacity to separate and refrigerate the samples rapidly will be sporadic. In addition, those measures provide only a cross-sectional snapshot of a woman's glucose status during a period of metabolic change.

In the NCS, biochemical measurement of maternal glucose metabolism early in pregnancy will be estimated by collection of serum for hemoglobin A1c. Hemoglobin A1c provides an integrated measure of maternal glucose levels over 6-10 weeks and will reflect exposures in the periconceptional period as well as during early embryogenesis. The analyte is stable for several days at room temperature, and thus is suitable for collection in the field. It does not, however, allow for assessment of subclinical impairment of glucose metabolism. In addition, the possibility of obtaining fasting specimens for glucose and insulin analysis exists for at least a sub-sample of the NCS population, depending on the characteristics of the individual study sites.

In addition to the biochemical measures obtained directly by the NCS, clinical reports of maternal OGTT results and fasting glucose tests will be obtained during the perinatal chart review. Maternal and family diagnoses of diabetes will be obtained via questionnaire.

9.5.4 Endocrine Markers

Two endocrine exposure measures are of specific interest to the NCS: maternal thyroid hormone, and cortisol in both the mother and the child.

Maternal hypothyroidism is associated with sub-optimal neurodevelopment in exposed offspring. The potential influence of subclinical hypothyroidism, especially as it relates to maternal exposure to hormonally active compounds (primarily some of the persistent organic chemicals discussed earlier), on subsequent health is not known.

Blood for assessment of maternal thyroid stimulating hormone (TSH) and thyroxine levels will be obtained prior to pregnancy in the pre-conception cohort, enabling periconceptional estimation of fetal exposure. Thyroid measures will be obtained from all women at the first trimester home visit and at the third trimester clinic visit. Cord or neonatal heel stick blood will be available for late fetal assessment of thyroid status.

Maternal stress or response to stress, as measured through cortisol, may influence the development of the fetal immune system and lead to persistence of the TH2-type response associated with asthma and atopy in childhood. Whether this is due to the central effect of stress on the maternal HPA axis or to other mechanisms (e.g., maternal and fetal response to placental CRH) is not certain.

Cortisol measures may be performed in blood, saliva, or urine. Blood measurements of cortisol reflect total cortisol, including protein-bound cortisol. In saliva or urine, cortisol measures are believed to more accurately reflect the free, biologically active fraction of cortisol. Therefore measurements of cortisol are performed using multiple measures in saliva in a day or using a 24 hour urine collection.

The NCS will obtain multiple daily saliva specimens from the mother twice during pregnancy to capture the diurnal patterns in cortisol that enable characterization of stress response (see Appendix G). Maternal and paternal samples will be obtained at 6 months as a biological indicator of parental stress and depression, to be used in conjunction with assessments of child development, family process, and related domains. In early childhood, saliva samples will be attempted at the 6- and 12-month visits for evaluation of HPA activity. At each collection, three to four samples will be collected at specified times (on awakening, mid-day, evening) to check for diurnal patterns. Urine samples can be collected as an alternative to saliva if needed.

Maternal stress will be assessed via questionnaires at several time points during and after pregnancy and periodically throughout childhood (see Section 9.3.3) to enable comparison between reported or perceived stress and biologic measures of stress that may be affecting the fetus or affecting parenting efficacy after birth.

9.5.5 Parental Medical History

The mother's past and current medical history will be obtained during the first interview. Past history will include ascertainment of chronic disease, such as asthma or diabetes; frequent acute

disease, such as urinary tract infections; and mental health, such as depression or anxiety disorders. There will be a focus on factors potentially related to pregnancy outcome, including the mother's birth history (preterm birth, birth weight, plurality, prior pregnancies, and outcomes), and reproductive history (age of first menstruation, menstrual cycle, doctor visits, and normal health care providers). Additional information about the current pregnancy (due date, hospital, pregnancy-related conditions, and illness during the periconceptional period or early pregnancy) will be obtained and updated throughout the pregnancy. Data on use of fertility services will also be collected early in pregnancy. Maternal family history, including the histories of parents and siblings, will be obtained as well, with a focus on chronic and mental diseases.

The identified biological father will also complete a medical history and a family medical history early in pregnancy.

Information about maternal doctor visits, diagnoses, and other medical events will be collected in a diary prior to and throughout pregnancy, and in a health care visit log. After birth, the focus will shift to the child's medical conditions, doctor visits, injuries, and use of car seats. Structured information regarding contacts with the health system will be recorded in a health care visit log.

9.5.6 Health Behaviors and Status

Starting in pregnancy, measures of health behavior and health status in the NCS will be taken or adapted from those commonly used in other epidemiologic studies. The domains include use of tobacco products, alcohol consumption, substance abuse, diet, and physical activity. In addition to these, car safety seat use, maternal sleep habits, maternal douching during pregnancy, the presence of breast implants, eating disorders, dental health, parental and child health history, and documentation of clinical encounters (for unexpected events) will be recorded. Multiple longitudinal measurements of many of these exposures are required due to their variability between time points and the cumulative effect of behaviors on long-term health of the child.

Diet: Within the NCS, dietary intake of the mother during pregnancy in conjunction with the child's diet is considered the major factor influencing nutritional status and is considered a potential source of chemical (primarily pesticide and metals such as mercury) exposure. If issues of burden and cost were not considerations, a minimum of four 24-hour diet recalls or two sets of four-day food records would be collected on the mother or child at each measurement point, following the precedent of more focused studies (U.S. Department of Agriculture, Rhodes et al., 2004). These methods also include coding for food preparation, an important source of toxicant exposure. The 24-hour method underreports less than other methods (Subar et al., 2003) and minimizes recall bias. However, a single 24-hour report is not representative of an individual's total diet and should not be used to estimate actual diet (Research Council, 1986). As a result, recall over multiple days is needed to assess an individual's usual intake.

For these reasons, the NCS has selected a self-administered Food Frequency Questionnaire (FFQ) as the primary method of collecting dietary exposure data for mother and child. This approach is the most commonly used assessment method in large epidemiological cohort studies. FFQs ask respondents to report their usual frequency of consumption of each food from a list of foods for a specific period. Information is collected on frequency and sometimes portion size. The FFQ's major strength is its ability to estimate usual intake of foods during a long period of time (e.g., past week, month, or year). Because it is self-administered, it is relatively inexpensive and does not have to be completed during a Study visit. The FFQs will be augmented by a three-day checklist, and, for the child, will be supplemented with breastfeeding and formula questions at 6 and 12 months.

Of the validated FFQs used in epidemiological research, the NCI Diet History Questionnaire (DHQ) has been chosen for the NCS because it is a public use instrument; the paper questionnaire completed by the participant can be optically scanned; it can be modified to add additional foods and questions of interest to NCS; and it can be linked to the major exposure databases (Total Diet Study; USDA/EPA Pesticide Database Program; Dietary Exposure Assessment Module; Dietary Exposure Potential Model). The DHQ will be administered to NCS mothers at preconception, twice during pregnancy, and at one month after birth for lactating mothers. The NCS will also use the Harvard Service Food Frequency Questionnaire (a proxy form, generally completed by the mother) to collect information on children at 18 months and 36 months (Blum et al., 1993; Gilman, ongoing; Welsh et al., 2005).

As an adjunct to the FFQ, a self-administered three-day checklist will be used to collect information about the current diet before and during pregnancy, and the early post-natal period. For the child, a three-day checklist will be used at 6, 12, 18, and 36 months. Other dietary instruments include feeding forms for children at 1, 6, and 12 months. These self-administered forms collect information about frequency and quantity of breast and formula feeding, and types of formulas. They also include questions on initiation of solid foods, preparation of formula and bottles, and use of commercial baby foods. They have been adapted from the FDA Infant Feeding Practices Study II (http://www.cdc.gov/ifps/). The child's diet will continue to be measured longitudinally throughout development.

Physical activity of the mother will be measured using the International Physical Activity Questionnaire (IPAQ)–Short, Last Seven Days. This instrument allows the calculation of total physical activity in metabolic equivalents (METS), which can then be used to compare different levels and types of activity within the NCS, and also with the numerous other studies that use the IPAQ. The IPAQ is recommended for monitoring population levels of physical activity globally for those ages 18-69. The mother's physical activity will be assessed prior to pregnancy and again early in pregnancy.

Information allowing the estimation of the child's physical activity will be collected at 6 and 12 months through the questions used to assess his or her usual activities and developmental status. Time-activity diaries will also be employed starting at 12 months. Starting at 36 months, the use of accelerometry may be attempted, though the protocol to be used for those measurements has not yet been determined. Physical activity will be measured throughout development and the specific measures will be determined starting two years before each wave of data collection.

Tobacco use: Maternal use of products containing tobacco will be ascertained throughout pregnancy and updated after the child is born. Questions about tobacco use, including prior and current usage and type of product used, are adapted from NHANES and the National Survey of Family Growth. Tobacco use will also be assessed through diary entries completed by women prior to and throughout pregnancy. In addition, cotinine will be analyzed from urine, hair, or blood samples drawn from the mother before and during pregnancy. Paternal use of tobacco and tobacco use by other household members will be also be ascertained during pregnancy and infancy by similar methods. Maternal tobacco use during pregnancy has been reported in association with Attention Deficit Hyperactivity Disorder.

The child's actual exposure to tobacco in utero and after birth will be estimated by measuring cotinine in the cord blood or heel stick, and in urine at 6 and 12 months. In addition to the questionnaire and biologic samples, house dust collected at the home visits can be analyzed for nicotine to allow further categorization of potential tobacco exposure.

Measures of environmental tobacco smoke will be made throughout childhood, and the child's own use of tobacco in adolescence will be carefully investigated.

Alcohol use and abuse: Use of alcohol by the mother will be ascertained by questionnaires before and during pregnancy. Relevant questions were adapted from other major epidemiologic studies including NHANES, World Health Organization–ASSIST, and the Coronary Artery Risk Development in Young Adults (CARDIA) Study.

Questions on alcohol use will include amount, frequency, and type of alcohol, both with regard to the year prior to the time woman knew she was pregnant, and with regard to current use (during early pregnancy and late pregnancy). Excessive use by the mother will also be assessed after child's birth.

Use of prescription drugs in ways other than those prescribed by a doctor will be obtained for the year prior to the time woman knew she was pregnant, and during pregnancy.

Medications and supplements: Use of prescription medicines, over-the-counter medicines, supplements, and alternative medicines will be assessed prior to and throughout pregnancy by direct observation of medicine bottles by the NCS data collector during in-person contacts. This technique has been used by many epidemiologic studies (including NHANES) and provides an accurate inventory of medications in use. Prior to the visit, the participant is asked to gather bottles of all medicines she is currently taking. This can easily be done when the visit occurs in the home; for clinic visits, the participant will be asked to bring the bottles to the study clinic. By reviewing the bottle, the interviewer is able to reliably record the name, strength, dosage, and form of each medication.

Use of over-the-counter and prescription drug, dietary and pharmaceutical supplements, and herbal and alternative medications will also be ascertained via the maternal and child questionnaires. Prescription information will also be collected in the health care visit logs. An important source of fetal exposure to medication will be the abstraction of the maternal prenatal, labor, and delivery records at the birth hospital.

Abuse of drugs that are prescribed by a doctor will be ascertained by questionnaire using a single measure which groups classes of drugs (e.g., sedatives, tranquilizers, analgesics, etc.) During pregnancy, two time periods are covered – the year before the woman knew she was pregnant and currently. This measure was taken from the Composite International Diagnostic Interview (CIDI), drug module.

Illicit drugs: Self-report of illicit drugs will be obtained from the mother before and during pregnancy and after birth. A single measure has been selected to ask about major categories of street drugs, including amphetamines, marijuana, cocaine, inhalants, hallucinogens, and opioids. This measure has been used previously for CIDI and (World Health Organization— Alcohol, Smoking, and Substance Involvement Screening Test [WHO-ASSIST]). Drug screening of biologic samples (blood, cord blood, and urine) can also be performed. Use of illicit drugs by the child will also be assessed.

9.5.7 Other Health-related Behaviors and Status

The dental health assessment by questionnaire includes questions adapted from NHANES regarding routine cleanings, gum health, past dental procedures, dental problems, and use of dental rinse products. Dental health questions will be asked prior to and throughout pregnancy. Performance of a clinical diagnostic periodontal and dental examination was initially considered for inclusion, but is not practical given the NCS visit schedule and geographic distribution of the study population.

Maternal and child sleep habits will be assessed prior to pregnancy, throughout pregnancy, and during early childhood. Questions include amount of time sleeping at night, amount of time sleeping

during the day, and sleep apnea during the past week (from the National Heart, Lung, and Blood Institute, Assessing Child and Maternal Sleep in the Early Years).

Other maternal health related behaviors to be collected by interview include current and past eating disorders, information about current and past breast implants, and frequency of douching and type of douche product (prior to pregnancy and during early pregnancy).

9.6 Genetic Measures

The longitudinal design and scope of the NCS provide vital resources to help answer many questions related to the role of genetics and genomics in the health of our nation's children. The size of this cohort, and the fact that exposures are measured during pregnancy and pre-pregnancy (in a sub-sample of the cohort), will also provide a unique opportunity to investigate the combined effect of genotype and exposure on structural and functional properties of the brain and on other organ systems during development. In this context, the ability of the study to investigate fetal/mother interaction during pregnancy will be especially important.

Venous whole blood samples will be collected from the mother and father to obtain genomic DNA and to extract and store peripheral blood mononuclear cells (PBMCs) for later transformation into cell lines. Genomic DNA from whole blood is the gold standard for most genetic studies, especially those involving genomic variation of candidate genes, and will be obtained in the NCS. Genomic DNA can also be utilized for whole genome genotyping studies (linkage or association), sequencing, epigenetic studies, and assessing change in genetic material over time (National Children's Study Workshop, 2004; Wallace, 2007). Cord blood will be collected at birth to obtain a sample of germ line DNA and RNA, and to extract and store PBMCs for later transformation into cell lines. A heel spot will also be obtained from the child at birth to confirm the purity of the cord blood sample. In the cases where blood cannot be drawn, saliva will be collected to extract DNA. A summary of assessment approaches for genetics appears in Table 9-5.

Table 9-5. Summary of NCS Genetics Assessment Approaches

Approach	Types of samples	Target components (measures)
Biomarkers	Whole blood	Genomic DNA: SNPs & haplotypes, gene expression, DNA adducts, nucleotide sequences (if/when economically feasible) Epigenetics RNA Mitochondrial DNA: haplogroups, somatic mutations
	Peripheral blood mononuclear cells	Cell lines, epigenetics
	Cord blood	Imprinting, epigenetics
	Saliva	DNA (if blood draw refused)

9.6.1 Genomic DNA

The HapMap Project has shown that approximately 80 percent of recombination occurs in about 15 percent of the genome; the project has reduced the task of measuring millions of single nucleotide polymorphisms or SNPs by using linkage disequilibrium to identify a reduced set of tag SNPs that captures variation throughout the genome (Gibbs & Singleton, 2006). The NCS will obtain genetic

samples from multiple family members across a broad sample of participants, and will therefore give investigators the chance to perform both linkage and association studies which use both population and family based study designs (Laird & Lange, 2006). Genome wide-association studies (GWAS) have emerged as a robust and unique approach to identifying multiple interacting disease susceptibility genes and their respective pathways (Keith, 2007; Yeager et al., 2007). Studies of genomic variation in NCS children can contribute information not only about the relation of genetic variants to disease risk (e.g., susceptibility, severity, prognosis) and the interaction of genotype with environmental risk factors (e.g., the 5-HTTLPR serotonin transporter gene, early life stress and alcoholism), but also about the response to therapeutics (Duff, 2006; Keith, 2007; Kelsoe, 2004; Laird & Lange, 2006; Laird, 2005; Reich & Patterson, 2005; Wallace, 2007; Yeager et al., 2007). The size of the NCS cohort helps protect linkage and association methods from population stratification, allowing case control association studies to be more successful and giving NCS investigators a variety of avenues for research.

9.6.2 DNA Modifications

Many DNA modifications and alterations have been shown to be caused by environmental exposures (Flato, Hemminki, Thunberg, & Georgellis, 1996; Kiyohara & Yoshimasu, 2007). If these alterations are not repaired appropriately by the body's DNA repair mechanisms, genetic instability and mutations can result that contribute to increased disease risk (Flato et al., 1996; Kiyohara & Yoshimasu, 2007). Genetic variations in DNA repair genes have been shown to impact DNA repair capacity, ultimately impacting disease susceptibility (Kiyohara & Yoshimasu, 2007). In addition, chemical compounds can attach to DNA molecules to form adducts which are often studied as molecular measures of exposure (Verdina, 2006). Whether a DNA adduct has biological significance depends on several factors including the type of adduct formed and the rate of DNA repair. Modifications are time dependent, and thus the multiple, repeated blood collections planned in the NCS will help to identify possible genetic modifications resulting from environmental influences and exposures (Flato et al., 1996). The identification of molecular biomarkers such as DNA adducts, in combination with high-throughput genotyping techniques to identify polymorphisms in DNA repair and other genes, will facilitate the characterization of exposures mediating disease pathways and related outcomes in the NCS sample.

9.6.3 Epigenetics and Epigenomics

The design of NCS is ideal for studying epigenetic effects, such as DNA methylation and histone modifications, which result in changes in gene expression that (though maintained through meiosis and/or mitosis) do not involve alterations in DNA sequence (Rodenhiser & Mann, 2006). Epigenetics is defined as the study of heritable changes in gene expression and function that occur through alterations in the chromatin structure, ultimately impacting transcriptional control of genes (Rodenhiser & Mann, 2006). The collection of genetic samples from the mother, father, and child trio, and possibly from other family members, will also provide a strong opportunity to study epigenetic modifications to genomic DNA (Laird & Lange, 2006). Epigenetic changes can provide insight into how aspects of the environment, such as chemical or psychosocial exposures, affect gene regulation (Anway & Skinner, 2006). The epigenetic influence of some exposures is time dependent, having a stronger influence at certain stages of development than at others. The multiple measures of exposures, biospecimens, and outcomes in the NCS will facilitate investigation of these critical exposure windows on the epigenome. Epigenomics promises a unique perspective of the genome due to the ability to identify and detect quantitative modifications and alterations outside of genes. Emerging high-throughput technologies, such as microarray analysis, will facilitate a reproducible and quantitative approach to epigenomic analyses (Callinan & Feinberg, 2006). A good example of the type of epigenetic change relevant to the NCS would be dietary influences on gene expression, such as folate deficiency, which can

influence DNA methylation in pregnant mothers, predisposing their children to several complex diseases including anemia (Donnelly, 2001).

9.6.4 Mitochondrial DNA

Disturbances in mitochondrial DNA (mtDNA) metabolism have been implicated in developmental delay, mental retardation, dementia, seizures, neuro-psychiatric disturbances, migraines, strokes in the young, and movement disorders (Naviaux, 2000). The spectrum of diseases associated with mitochondrial dysfunction or variation in mtDNA is expanding into disorders such as autism (Graf et al., 2000) and diabetes mellitus type II (Mogensen et al., 2007; Weijers & Bekedam, 2007; Fuku et al., 2007), and may play a role in susceptibility to some environmental exposures.

Several characteristics of mtDNA are distinct from nuclear DNA and make mtDNA an interesting biomarker of disease and exposure. Mitochondrial DNA is exclusively maternally inherited. Therefore, mtDNA sequences are not altered by recombination as passed from generation to generation, but through the accumulation of mtDNA mutations along female lineages (Brandon et al., 2006). In the NCS, because DNA will be obtained from both mothers and children, it will be possible to track some of the lineage.

In addition to maternal inheritance, and unlike inheritance of nuclear DNA that occurs in an all-or-none fashion, the frequency of transmission of mutated mtDNA is stochastic and may occur in a range of 0-100 percent transmission. This results in a mixture of normal and mutant DNA (Brandon et al., 2006; Gropman et al., 2004; Wallace, 2005; 2007). The percentage of mutated mtDNA transmitted may be associated with distinct phenotypes (Wallace, 2007). The large sample size of the NCS makes it well suited to examining the association of different percents of mutant mtDNA with disease phenotypes.

Moreover, mtDNA has a high mutation rate. mtDNA is particularly susceptible to DNA damage in comparison with nuclear DNA (Marcelino & Thilly, 1999; Masayesva et al., 2006; Yakes & Van, 1997) due to the lack of histones protecting the DNA and reduced efficiency of DNA repair (Kujoth, Bradshaw, Haroon, & Prolla, 2007; Penta, Johnson, Wachsman, & Copeland, 2001) when compared with nuclear DNA. The mitochondria produce a large amount of reactive oxygen species (ROS), which can damage DNA. As a result, mutations in mtDNA accumulate in post-mitotic cells of the body with age (Kujoth et al., 2007; Wallace, 2005). Because the NCS will obtain specimens at different times during development, it will be possible to track potential changes in mtDNA.

Mitochondrial DNA will be isolated from whole blood of NCS participants and obtained several times during development. In addition to isolating mtDNA from blood, in some studies it has been detected in urine (Fliss et al., 2000) and in saliva (Fliss et al., 2000; Masayesva et al., 2006). Both will be collected in NCS, and may be used for ancillary studies. Examination of changes in mtDNA will be performed by DNA sequencing or using genotyping technology. Another approach for high throughput sequencing of mtDNA, which will be explored by NCS, is the use of the MitoChip, an oligonucleotide microarray for rapid sequencing of the entire mitochondrial genome (Sui et al., 2006; Jakupciak et al., 2005).

9.6.5 RNA

Some recent studies examined the use of RNA obtained from whole blood or peripheral blood mononuclear cells (PBMCs) for use of expression profiling (Lampe et al., 2004; Whitney et al., 2003). In these studies, using both whole blood and isolated PBMCs, variation of gene expression profiles

were observed among individuals (Debey et al., 2004; Whitney et al., 2003) The variation of gene expression in healthy subjects was much smaller than the variation observed in individuals with cancer or bacterial infection (Whitney et al., 2003). This suggests that gene expression profiling of RNA obtained from blood is a possible biomarker of disease. Furthermore, gene expression patterns from isolated PBMCs may be altered by exposure, as suggested by the observation of a gene expression signature associated with tobacco smoking (Lampe et al., 2004). In the NCS, whole blood and PBMCs will be collected for studies of gene expression. RNA obtained from whole blood specimens represents several cell types, while PBMCs are only one type of cells. Therefore, these RNA sources may be used to address different questions relating to gene expression (Debey et al., 2004; Whitney et al., 2003).

One of the greatest challenges to this type of RNA analysis both from whole blood and PBMCs is that the samples tend to degrade quickly during collection and storage. Preserving RNA is vital since the stability of the RNA affects the analysis of gene expression, consequently preserving RNA with RNAase inhibitors is imperative. Commercially available blood collection tubes that reduce RNA degradation and additives to stabilize RNA exist but are expensive (Chai, Vassilakos, Lee, Wright, & Young, 2005; Pahl & Brune, 2002; Rainen et al., 2002). To improve stability in the NCS, RNA isolation from whole blood is planned at the central repository prior to long-term storage. RNA may also be isolated from cryopreserved PBMCs; one study observed high quality RNA extraction from PBMCs which were frozen for 15 months (Marteau, Mohr, Pfister, & Visvikis-Siest, 2005). Many issues related to stability will undoubtedly be solved during the next few years, but issues with respect to long term storage will be a challenge.

9.6.6 Cell Lines

Generating cell lines from collected specimens will provide a valuable resource for future studies. This is an expensive process, but if cell lines are generated, an almost unlimited supply of genetic material will be available to investigators for many types of future studies, including genetic and biochemical assays (Beck, Beiswanger, John, Satariano, & West, 2001; Hayes, Smith, Huang, Read, & Kopp, 2002). Cell lines and PBMCs also provide a source for the development of phenotypic assays. Such assays allow exploration of the function of entire biological pathways to determine if reduced efficiency of a particular pathway is associated with disease. These assays either examine enzyme activity or expression of particular proteins.

To utilize samples cost effectively, the NCS plans to isolate and cryopreserve the PBMCs within 30 hours of collection. PBMCs will then be transformed into cells in the future when relevant cases necessitating such transformation have been identified. Previous studies suggest that cryopreserved PBMCs may be stored for two years or more prior to transformation with high transformation efficiencies (Beck et al., 2001; Hayes et al., 2002; Kleeberger et al., 1999).

Chapter 10

Statistical Analysis Plan

10. STATISTICAL ANALYSIS PLAN

10.1 Introduction

10.1.1 Study Design

The design for the National Children's Study (NCS) is based on a nationally representative sample of about 100,000 births to be sampled in 105 geographic areas, called either primary sampling units (PSUs) or Study sites. The pregnancy status of all eligible women of child-bearing age in these areas will be monitored for 4 years, and prepregnancy survey data will be collected for those trying to get pregnant. All women living within the Study sites who become pregnant during the 4-year period will be enrolled in the Study as early in pregnancy as possible in order to measure in utero exposures. The pregnant women will be followed through birth; their children will be followed for 21 years. Throughout this period, the NCS will collect extensive data on a variety of health outcomes and environmental measures and social, demographic, economic, and neighborhood characteristics.

10.1.2 Objectives

The NCS is designed to address hypotheses developed over several years by a variety of stakeholders following a review of the current state of the art in the many areas related to child development and environmental exposures. These primary or "core" hypotheses relate to multiple diseases and developmental outcomes, including asthma, physical and neurological development, diabetes, adverse pregnancy outcomes, obesity, and behavior and mental health problems, such as autistic spectrum disorders.

The NCS will collect data on the children's exposure to chemical, physical, biological, psychosocial, and behavioral environments and their communities, child care, and schools. It will also collect data about the parents' workplaces concerning exposures that might affect their children and data on the children's health from their physicians. Thus, there will be multiple levels of data collection: individual, household, immediate neighborhood, community (e.g., community air quality), and county (e.g., schooling and sociodemographic characteristics).

The study will have the power to examine gene-environment interactions from a developmental perspective in a way that has not previously been done. The NCS will provide a rich source of data with which to investigate the genetic mechanisms associated with rare diseases such as autism; the quantitative contribution of genetic variation to common conditions such as obesity; and the impact of gene and environment interactions on complex diseases and conditions, such as asthma and depression. Multiple gene-environment and gene-gene interactions will play a key role, creating the need for highly complex, computer-intensive forms of analysis. An important goal of the NCS is to provide data to support such analyses.

10.1.3 Overview of the Chapter

This chapter describes statistical methods that will be employed in analyzing NCS data and important issues for these analyses. One primary consideration, of course, is the sample size and power that can be expected in the NCS. This is discussed in Section 10.2. In Section 10.3, we discuss a number of issues relevant to all statistical analysis of NCS data, such as design-based versus model-based

analysis, confounding, measurement error, and missing data. Section 10.4 illustrates the range of methods that will likely be used in analyzing NCS data, and Section 10.5 discusses analysis of genomic data.

10.2 Sample Size and Power

10.2.1 Overall Sample Size and Key Subgroups

As noted earlier, the overall sample size for the NCS is about 100,000 sampled children at birth. However, this number is expected to decrease by about 2 percent per year so that, for example, the sample size at age 18 will be reduced to about 69,000 children remaining in the study. Furthermore, the sample size will be smaller for some endpoints. For example, for schizophrenia, the sample size will be reduced because the postulated analyses require placental data and serum from early in pregnancy that are assumed to be available for only 80 percent of the sampled children.

In addition, some hypotheses apply to selected subgroups, defined by characteristics such as sex, race, ethnicity, living area, genotype, or combinations of these characteristics. Examples include the following outcomes: age at puberty, which requires separate analyses for boys and girls; asthma among breast-fed children; and IQ score among "at-risk" children. Subgroup sample sizes are often small, leading to substantially less power.

10.2.2 Impact of Complex Sample Design

The sample design for the NCS is a complex clustered design involving unequal selection probabilities, stratification, and multi-stage sampling. Complex sample designs, particularly clustered designs, have a substantial impact on standard errors and power. The impact of the complex design is measured by the design effect for a given survey estimate. A design effect greater than 1.0 indicates the estimate is less precise than the corresponding estimate computed from a simple random sample of the same size.

Much empirical research has shown that design effects for complex clustered sample designs are generally lower for analytic statistics, such as odds ratios and regression coefficients, than for descriptive statistics, such as means and proportions (see, for example, Kish 1995). The design effects for regression coefficients are discussed in Scott and Holt (1982). The estimates of power for the odds ratios presented in Section 10.2.3 incorporate an allowance for estimated design effects associated with the complex NCS sample design. For most of the calculations, the homogeneity of the exposures in the PSUs is assumed to be modest. However, for hypotheses relating to infant mortality and rate of developmental disabilities, the exposures are assumed to be highly homogeneous within PSUs. The reason for the high level of homogeneity in these cases is that the exposures of interest are neighborhood or community characteristics and policies that will be the same for all children in the neighborhood or community.

10.2.3 Power for Subgroups/Primary Objectives

In hypothesis-driven studies, there are two types of errors. A type I error (generally denoted as α) occurs when the null hypothesis is true but is rejected; a type II error (generally denoted as β) occurs when the null hypothesis is false but is not rejected. For example, if the null hypothesis is that a given factor is not associated with an outcome, then a type I error occurs when there is in fact no association but the study concludes that there is one. Type II error, failing to reject the null hypothesis when a given factor is actually associated with an outcome, is the complement of statistical power; thus,

the higher the power, the smaller the chance of making a type II error. While there are no universally accepted error rates, the values of $\alpha = 0.05$ and $\beta = 0.20$ (i.e., power = 80 percent), respectively, are most frequently used when designing studies.

A range of medically important outcomes will be used here to illustrate the ability of the NCS to test exposure-outcome associations involved in the primary hypotheses with power of 80 percent. These outcomes exhibit the range of prevalence that NCS outcomes are likely to have. While some outcomes are common, most are rare and some are very rare. Many of these outcomes are relevant for a single primary hypothesis, but some are relevant for more than one. For example, several hypotheses address different possible predictors of childhood asthma, including environmental factors, exposure to bacteria and microbial products, maternal stress during pregnancy, and diet. For each outcome, a set of different exposures is considered. In each case, power has been calculated for exposure prevalence of 1.0 percent, 2.5 percent, 5 percent, 25 percent, and 50 percent (this range is based on hypotheses developed for the NCS).

Using cerebral palsy (CP) as an example, the results on power displayed in Table 10-1 can be interpreted as follows: Since CP has a prevalence of about 0.2 percent in the general population, that is the rate to be expected in the NCS. Table 10-1 gives the odds ratio (OR) that can be detected with 80 percent power for exposures (i.e., risk factors) with a 5 percent significance level and a prevalence ranging from 1 percent to 50 percent. For very rare exposures (e.g., 1 percent), only those that have a dramatic impact on the occurrence of cerebral palsy (OR greater than or equal to 5.0) can be reliably detected in the NCS. However, for more common exposures, such as those with 5 percent prevalence or greater, factors with more modest effects (OR greater than or equal to 2.6) can be detected with 80 percent power.

Two simplifications were made in these power calculations. First, the analyses consider only the simple bivariate relationships between the exposures and outcomes without addressing the need to control for confounders. The inclusion of confounders likely results in a reduction in the power for detecting the effects of exposures, but often the reduction will be modest. Second, all outcomes and exposures are assumed to be dichotomous variables. This assumption is again made to simplify the table. In fact, most of the NCS outcomes and exposures will be continuous variables. As a result, the power estimates in the tables are likely to be conservative since dose-response analyses with continuous outcome and/or exposure variables would likely lead to greater power.

Table 10-1 displays the magnitude of the minimum odds ratios that can be detected with 80 percent power for the selected outcomes and the range of exposures for analyses. The sample sizes for Table 10-1 assumed to be the full sample for which data are available. As noted above, the sample available is reduced through attrition and, for some outcomes like schizophrenia, by availability of special data required for analysis. As Table 10-1 shows, the magnitude of the detectable odds ratio depends on the prevalence of both the outcome and the exposure. For a given outcome, the closer the prevalence of the exposed group is to 50 percent, the smaller the detectable odds ratio and the greater the power. Similarly, in general, the closer the prevalence of the outcome is to 50 percent, the smaller the detectable odds ratio, i.e., the detectable odds ratios are small when the exposure prevalence is reasonably high. All the ratios are less than 2 when the exposure prevalence is between 25 percent and 50 percent. The bold line in the tables separates the detectable odds ratios into those above and those below 2.

Table 10-1. Detectable Odds Ratio When Analyzing the Total Sample

Outcome	Age	Prevalence of outcome (%)	Prevalence of exposure				
			1%	3%	5%	25%	50%
Infant mortality*	1	0.7	6.01	3.87	2.95	1.97	1.94
Type I diabetes	18	0.2	5.71	3.72	2.86	1.93	1.89
Musculoskeletal defects	1	0.2	5.00	3.33	2.60	1.80	1.75
Cerebral palsy	1	0.2	5.00	3.33	2.60	1.80	1.75
Schizophrenia#	18	0.3	5.06	3.36	2.62	1.81	1.76
Nervous system defects	1	0.3	4.09	2.82	2.25	1.62	1.58
Metabolic syndrome	18	0.4	4.03	2.78	2.23	1.61	1.56
Autism spectrum disorder	4	0.4	3.66	2.57	2.09	1.54	1.49
Heart defects	1	0.6	3.03	2.21	1.84	1.42	1.38
Type 2 diabetes	18	1	2.75	2.05	1.73	1.36	1.32
Major birth defects	1	3.5	1.76	1.47	1.33	1.16	1.14
Adolescent aggressive behavior	18	4	1.82	1.50	1.35	1.17	1.15
Chronic physical aggression (CPA)	10	4	1.76	1.47	1.33	1.16	1.14
IQ score less than 75	18	5	1.73	1.45	1.31	1.16	1.14
Asthma	4	7.5	1.53	1.33	1.23	1.11	1.10
Neurocognitive development	12	8	1.55	1.34	1.24	1.12	1.10
Depression	18	8.3	1.57	1.35	1.25	1.12	1.11
Asthma	7	8.5	1.51	1.32	1.22	1.11	1.10
Neurodevelopmental disabilities	18	10	1.52	1.32	1.22	1.11	1.10
Preterm birth < 37 weeks	0	12	1.41	1.26	1.18	1.09	1.08
Asthma	18	12.5	1.47	1.29	1.20	1.10	1.09
Adverse pregnancy outcomes	0	15	1.38	1.23	1.16	1.08	1.07
Developmental disabilities*	18	17	1.92	1.54	1.37	1.18	1.16
Developmental disabilities	18	17	1.41	1.25	1.18	1.09	1.08
Obesity	12	17.1	1.39	1.24	1.17	1.08	1.07
IQ score less than 100	18	50	1.32	1.20	1.14	1.07	1.06

* The exposure for this hypothesis is a community rather than an individual level characteristic.
# This analysis is restricted to children for whom placental data and serum from early gestation are available (assumed 80 percent).

To illustrate the increase in the magnitudes of detectable odds ratios for subgroup analyses, Table 10-2 presents results comparable to those in Table 10-1, but with the sample size reduced to a 20 percent subgroup. The results in this table could be applied to case-control studies or other analyses based on subsets of the overall NCS sample. It is assumed that the geographic distribution of the subgroup is proportionate to the general population, which would generally be true in case-control studies and other subset analyses. The detectable odds ratio remains below 2 when the outcome prevalence is 3.5 percent or higher and the exposure prevalence is 5 percent or more, but for rarer outcomes and exposures, it exceeds 2. Many subgroups of interest will comprise less than 20 percent of the population and will thus have larger detectable odds ratios.

Table 10-2. Detectable Odds Ratio When Analyzing a 20 Percent Subsample

Outcome	Age	Prevalence of outcome (%)	Prevalence of exposure				
			1%	3%	5%	25%	50%
Infant mortality*	1	0.7	17.91	10.13	7.08	4.32	5.35
Type I diabetes	18	0.2	16.13	9.42	6.68	4.12	4.99
Musculoskeletal defects	1	0.2	13.44	7.96	5.69	3.50	3.93
Cerebral palsy	1	0.2	13.44	7.96	5.69	3.50	3.93
Schizophrenia#	18	0.3	13.73	8.08	5.77	3.54	3.99
Nervous system defects	1	0.3	10.23	6.19	4.51	2.81	2.93
Metabolic syndrome	18	0.4	10.04	6.07	4.42	2.76	2.86
Autism spectrum disorder	4	0.4	8.77	5.39	3.97	2.51	2.55
Heart defects	1	0.6	6.73	4.26	3.22	2.11	2.08
Type II diabetes	18	1	5.87	3.78	2.89	1.94	1.90
Major birth defects	1	3.5	2.96	2.15	1.79	1.39	1.35
Adolescent aggressive behavior	18	4	3.13	2.24	1.85	1.42	1.38
Chronic physical aggression (CPA)	10	4	2.97	2.16	1.80	1.39	1.35
IQ score less than 75	18	5	2.89	2.10	1.76	1.37	1.33
Asthma	4	7.5	2.35	1.80	1.55	1.27	1.24
Neurocognitive development	12	8	2.40	1.83	1.57	1.28	1.25
Depression	18	8.3	2.46	1.85	1.59	1.29	1.25
Asthma	7	8.5	2.30	1.77	1.53	1.26	1.23
Neurodevelopmental disabilities	18	10	2.33	1.78	1.54	1.26	1.23
Asthma	18	12.5	2.21	1.71	1.49	1.24	1.21
Preterm birth < 37 weeks	0	12	2.04	1.62	1.43	1.21	1.18
Adverse pregnancy outcomes	0	15	1.95	1.56	1.39	1.19	1.17
Developmental disabilities*	18	17	3.79	2.46	1.96	1.44	1.39
Developmental disabilities	18	17	2.07	1.62	1.43	1.21	1.18
Obesity	12	17.1	2.00	1.59	1.40	1.20	1.17
IQ score less than 100	18	50	1.90	1.49	1.33	1.15	1.13

* The exposure for this hypothesis is a community rather than an individual level characteristic.
# This analysis is restricted to children for whom placental data and serum from early gestation are available (assumed 80 percent).

10.3 Statistical Inference

10.3.1 Design-Based vs. Model-Based Inference

Statistical theory provides the basis for drawing inferences about a population based on a sample taken from that population. One approach to statistical inference that could be applied when analyzing NCS data is based on the sample design (design-based inference), i.e., the randomized procedures used to select the sample. An alternative approach is based on a statistical model that the underlying data are assumed to follow (model-based inference). Design-based inference provides the basis for most published descriptive estimates. However, model-based inference is often used when statistical methods are more complex. This section discusses these two analytical frameworks.

10.3.1.1 Design-Based (Randomization) Inference

In design-based inference, a randomly selected sample is used to estimate parameters that would have been obtained had all members of the population under study been included in the sample and provided data. These parameters may be termed census parameters (see, for example, Chambers & Skinner, 2003; Kalton, 2002). A given individual's data are considered fixed, however. The randomization comes about through the sampling used to select the individual. The statistical theory for the designed-based approach to inference from population-based survey data was developed in the late 1940s and discussions of this topic are available from many sources (e.g., see Cochran, 1977; Kish, 1965).

In regression analysis, the census parameters to be estimated consist of the census regression coefficients and the census squared multiple correlation coefficient. If the regression model is correctly specified and the population is large, the census parameters would be virtually the same as the model-based parameters. However, when the model is not correctly specified, the census parameters will differ from the model-based parameters. In this case, the model-based parameters are problematic, but the census parameters are still interpretable (at least under mild misspecification). The census parameters provide the best fitting model of the given structure for the population under study. In that sense, the design-based approach is somewhat robust.

There are two distinctive features of design-based inference: the need to use sampling weights when analyzing the data to estimate the census parameters and the need to take the sample design into account in estimating the standard error of estimates derived from sample data. The weights reflect the unequal selection probabilities with which sample units are selected and also weighting adjustments to compensate for nonresponse and noncoverage and to calibrate the sample to conform to known population distributions. Standard errors, p-values, and confidence intervals for survey estimates must be calculated using special procedures that reflect both sampling weights and any stratification and clustering used in the sample design.

The robustness gained from using weights in making sample estimates of census parameters comes at a price of a loss in precision as compared with correctly specified model-based estimates. With large samples and limited variation in the weights, that loss of precision is generally acceptable. There are, however, cases when the loss of precision is very large, such as when units are sampled with very unequal selection probabilities. In such cases, alternative estimation approaches may be required, for example, incorporating the sample design features into the analytic model (see, for example, Korn & Graubard, 1999, with examples from health surveys, and Chambers & Skinner, 2003).

10.3.1.2 Model-Based Inference

Model-based inference assumes a model for the population data *Y* as a function of a set of parameters θ. One version of this approach is superpopulation modeling (Royall, 1970; Thompson, 1988) where values of θ are considered fixed, and the observed population values are assumed to be drawn from a superpopulation whose distribution is given by $f(Y \mid \theta)$. Inferences about θ are based on the joint distribution of *Y* and the sampling mechanism *S*.

An alternative modeling procedure is Bayesian population inference (Little, 2004). As in design-based approaches, Bayesian population inference focuses on population quantities of interest $Q(Y)$. However, inference is made about $Q(Y)$ by considering the marginal posterior predictive distribution (Ericson, 1969; Holt & Smith, 1979; Skinner et al., 1989), which requires postulating a prior

distribution for the model parameters $p(\theta)$ in addition to the model for the data. This is similar to the missing data formulation in which all of the population not observed is considered to be missing and values are multiply imputed via the posterior predictive distribution of the data (Little & Rubin, 2002), although in practice the actual step of imputing values for the entire population can usually be avoided. Probability samples that are "noninformative" in the sense of Rubin (1987) in that the distribution of *Y* and *S* are independent (possibly conditional on fixed covariates *X*) so that the parameters θ and ϕ that govern the data and sampling mechanisms are distinct, allow inference to be made using a posterior predictive distribution based only on the model for the data. This is equivalent to the ignorable missingness assumption (see Section 10.3.4.1 for a discussion of this assumption) that allows inference about θ to be made conditional on observed data in item-missingness situations. However, to maintain the noninformative sampling assumption, the model must be formulated in a fashion that accounts for the sample design. Thus, for example, models being utilized in sample designs with unequal probabilities of selection might stratify based on the probability of selection to account for any associations between the parameters of interest and the probability of selection.

The model-based approach provides a framework in which point estimation and inference can be made in the same fashion as in other areas of statistics. As discussed in the previous section, the greatest disadvantage of the model-based approach is that, if the model is seriously misspecified, it can yield inferences that are worse—perhaps much worse—than design-based analysis. Careful model development and consideration of how and why models are likely to fail can serve as some protection against this outcome.

10.3.2 Confounding and Mediating Variables

Since the NCS is an observational study and not a randomized trial, the main challenge to making causal inferences from NCS data will be to control for confounding variables. Confounding variables are factors related both to an outcome variable and to exposure variables that are being evaluated as risk factors for that outcome, but that are not themselves dependent on the risk factors. The relationship between potential confounders and the outcome variable is not itself of analytical interest. However, the validity of estimated effects of exposures obtained from analyses depends critically on the inclusion of all the important confounders in the analysis.

When choosing potential confounders to be controlled for in an analysis, care must be taken to distinguish them from mediators. Confounders and mediators are each related to both the exposure and the outcome under study. However, confounders are causally prior to the exposure whereas mediators are on the causal path between the exposure and the outcome. Controlling for mediators will lead to a reduced or nonexistent relationship between the exposure and the outcome, thus providing a false impression of the full effect of the exposure on the outcome.

Confounders should also be distinguished from effect modifiers, sometimes called moderators. Effect modifiers partition an independent variable into subgroups where the effects of the independent variable on the dependent variable differ within each subgroup (Baron & Kenny, 1986). For example, Simons and Wood (2004) found that response to ozone exposure varies both by age and gender, with older persons (and particularly older women) experiencing less reduction in FEV_1 than younger persons. Thus, age and gender are effect modifiers for response to ozone exposure.

Appropriate control for confounders, whether by regression methods or propensity scoring, is essential with the NCS data. The NCS will collect information on a wide range of covariates that may be considered as potential confounders for a given analysis. In studying the possible effects of

environmental pollutants on asthma and wheezing, for example, there are a number of confounding variables related to environmental and genetic factors as well as to the risk of asthma and wheezing in children. In general, the approach utilized will be to review the scientific literature that describes previously observed factors associated with environmental and genetic factors and the increased risk of asthma and wheezing in order to select a set of covariates that are potential confounders for a specific analysis. In the asthma example, potential confounders in an analysis of the possible effects of environmental pollutants and genetic variation on asthma severity might include maternal gestational factors, such as premature birth and stress and infection during pregnancy; childhood infections; diet and nutrition; socioeconomic variables, such as parents' education, household composition, and housing characteristics; demographic characteristics such as race/ethnicity; access to health care; and so forth.

Sections 10.4.2.1 and 10.4.2.2 discuss linear and nonlinear regression and propensity scoring as methods for controlling for confounding variables. Matching is another method used to control for confounding variables. In matching, the individuals in the comparison group are selected to match the target group on a potentially confounding variable. This holds the effect of the confounding variable constant across groups in analyses. For example, in a study of the influence of prenatal drug exposure on children's cognitive development, the nonexposed comparison group would need to be matched to the drug-exposed target group on premature birth status to rule out a potential alternate explanation for cognitive delays in the drug-exposed group. Case-control studies, which represent a particular example of matching, are discussed in Section 10.4.6.

10.3.3 Measurement Error

10.3.3.1 Impact of Measurement Error

The role of measurement error in the analysis of epidemiologic data (both environmental and other study data) is multifaceted. As with any other types of data, there is the potential for bias and increased uncertainty in predicting outcomes when outcomes or covariates are measured with error. Measurement error in environmental exposures often results from data collection decisions. For example, there is potential bias and increased uncertainty in ecological designs where the required individual-level exposures are measured at the population or group level rather than at the individual level.

Individual measurements for each subject at each time period may contain measurement errors. The extent of these errors, such as those caused by equipment limitations, may be constant across subjects and time, but they may also vary due to collection or processing methods across laboratories or study sites, particularly for measures based on environmental samples or biospecimens. A further complication is that NCS analyses will not be restricted to estimating mean levels or correlations. Some analyses will require sampled individuals to be classified according to whether or not they have been exposed to chemical levels above certain cutoffs; other analyses will want to use continuous measurements to investigate whether threshold levels are the same at different developmental stages.

The effects of measurement error, which depend on the measurement error distribution (Carroll et al., 1995), that are possible include: (1) attenuation of a regression coefficient or other effect measure to the null; (2) hidden effects; and (3) a sign reversal in estimated coefficients. Thus, measurement errors can introduce both variability and bias into data analysis and must be accounted for.

10.3.3.2 Types of Measurement Error

The two general types of covariate measurement error are classical and Berkson error. Let X represent the true covariate measure that cannot be observed for all study participants. If X is fixed and the surrogate measure W varies due to error, then the classical measurement error model is appropriate, $W = X + U$, where U represents measurement error. For example, the biological samples of phthalates obtained from blood/urine, cord blood, infant urine, and meconium samples are potentially measured with classical measurement error. Conversely, if W is fixed and X varies due to error, the regression calibration or Berkson model is appropriate, $X = W + U$. For example, if the variable of interest is the actual amount of chemical absorbed by the body, the measurement of the chemical level in drinking water or air particles may be the fixed surrogate with the true level absorbed by the body varying as a function of the surrogate.

10.3.3.3 Assessing Measurement Error

There are several sources of data that can be used to evaluate the extent of measurement error. In some cases, study data can be validated for a subsample of cases. For example, it may be possible to obtain more accurate observations for a subset of the primary data or, at an aggregate level, from external sources. For air quality measurements, it is often too expensive to take a personal measurement for every study participant. It is more reasonable to randomly select a subset of the sample to measure personal air quality and collect more general measures such as room air quality for the entire study. The personal measurements can then be used to assess the extent of measurement error due to using room air data.

With replication or reliability assessment, multiple measures of the surrogate variable are observed via internal or external sources; the variation in the replicated measurements gives an indication of extent of variable measurement error.

Another approach uses instrumental variables. An instrumental variable must be (1) correlated with X, the covariate being measured; (2) independent of $W - X$ (i.e., measurement error: the true value minus observed value); and (3) independent of the outcome (Y) given X and any additional covariates that are measured without error (Z) (Carroll et al., 1995). For example, in the Faroese Mercury Study of a birth cohort of children, neither validation data nor replication data were available to estimate the cord blood mercury measurement error (Budtz-Jorgensen, et al., 2003). Instead, secondary exposure variables such as the concentration in maternal hair and the average number of whale dinners per month were used as instrumental variables.

10.3.3.4 Modeling Approaches

Regression calibration is essentially the replacement of the true covariate X by the regression of X on (Z, W) using replication, validation, or instrumental data (Carroll & Stefanski, 1990). It follows an algorithm of the following three steps: (1) use validation, replication, or instrumental data to estimate the regression of X on (Z, W); (2) replace the unobserved X by the estimate from step 1 and rerun the standard analysis to obtain parameter estimates; and (3) adjust the resulting standard errors to account for the estimation. In some cases, a simulation extrapolation approach can be used if validation or replication data are not available to model the calibration function. Heuristically, this approach is a self-contained simulation study that illustrates the effect of measurement error on parameter estimates (Carroll et al., 1995). There are commands available in Stata version 8 as well as macros for SAS that will fit generalized linear models when one or more covariates are measured with error.

In the context of structural equations models, it has been shown that latent variable models can be used to adjust for measurement error in the predictor and response with multiple measures on all subjects (Palta & Lin, 1999).

10.3.4 Missing Data

This section discusses types of missing data that will be encountered in the NCS, and methods that can be used either to adjust for them or to analyze data in the presence of missing data. In any survey, there are data losses due to noncoverage and nonresponse. Noncoverage occurs when individuals are missed in the listing process resulting in some members of the target population having no chance of selection. Total nonresponse (also called unit nonresponse) refers to eligible individuals who are sampled but do not provide any usable survey data. Item nonresponse refers to missing data items for eligible individuals who participate in the study and provide most of the required survey data. Partial nonresponse refers to eligible individuals who are sampled for the study but who provide only a portion of the survey data. This can occur, for example, when data collection involves multiple components (e.g., lab tests, questionnaires, etc.). Wave nonresponse occurs in longitudinal studies in which a sampled individual fails to provide data for one or more of the required waves of data collection. This type of nonresponse can be due to attrition, in which an individual who participated in early waves of data collection drops out of all subsequent waves. Wave nonresponse can also be intermittent rather than attritive, where a participant misses one or more waves of data collection but returns in a subsequent wave.

10.3.4.1 The Missingness Mechanism

When developing methods to account for missing data, it is important to evaluate the process that gave rise to the missing values. This process is called the missingness mechanism. Rubin (1976) and Little and Rubin (2002) define a typology of missingness mechanisms. Data are missing completely at random (MCAR) if the missing data are essentially a simple random sample of the underlying complete data. MCAR is unlikely to hold across an entire sample, but it may hold within strata or classes defined by race, sex, geographic location, or other variables.

The second mechanism is called missing at random (MAR). Data are said to be MAR if the probability that an observation is missing depends on the underlying complete data only through elements of the data that are fully observed. For example, if the probability that a subject drops out depends on classes defined by race, sex, or geographic location and class membership is known for all sampled persons, then the data are MAR.

The third type of missingness is the nonignorable (NI) mechanism. When data are NI, the probability of missingness depends on unobserved data even after adjusting for all observed data. With NI data, the application of standard approaches for handling missing data in the analysis are not valid. Since it is not possible to distinguish NI from MAR using observed data, the only way to identify NI missing data with any confidence is to gather the missing data from a fraction of those not responding. Thus, attempts to model NI missing data are generally speculative and have a limited role in applications.

10.3.4.2 Compensating for Missing Data in Design-Based Analysis

Design-based methods to compensate for missing data consist primarily of weight adjustment and imputation. The following sections describe methods that will be used to weight the NCS data as well as alternative methods for compensating for missing data under model-based inference. The model-based approach for handling missing data in the National Children's Study is discussed in Section 10.3.4.3.

Weighting adjustments

The primary method of compensating for unit nonresponse in survey data consists of adjusting the sampling weights. The initial sampling weight for each respondent is the inverse of the original selection probability for that respondent. These initial weights (often called base weights) can be adjusted for unit nonresponse and, in many cases, noncoverage using methods described below (see Brick & Kalton, 1996, for example, for a more detailed discussion). The NCS will use these procedures to adjust for unit nonresponse.

To compensate for nonresponse, adjustment factors are calculated within selected weighting classes formed by demographic or other data. These data must be available for both respondents and nonrespondents. The adjustment factors are then used to inflate the base weights. The weighting classes are typically based on information from the sampling frame. The underlying assumption is that the nonrespondents are missing completely at random (MCAR) within the weighting classes.

Adjustments for noncoverage are based on external data sources, typically the Census of population or, in this case, of National Children's Study birth certificate counts. The adjustment process consists of calibrating nonresponse adjusted weights so that sample estimates of key characteristics conform to the known population characteristics from the external source. This calibration compensates for noncoverage and it also reduces variance of estimates associated with the characteristics involved in the calibration. Birth certificate data are a likely external data source that can be used for making nonconvergence adjustments in the NCS. These data can, for instance, provide data on the numbers of births to mothers resident in a county and on the characteristics of those births (e.g., birth weight, APGAR results) and of the families (e.g., mother's age, race, education).

Imputation

Imputation is widely used in survey research to assign values to missing survey items and thus compensate for item nonresponse. The imputed values are derived using data from other items available for the respondent that serve as predictors of the missing values. The "hot deck" method is the simplest and probably most frequently used imputation method. In this method, missing data are assigned values from another respondent who is judged to have similar characteristics. For example, missing income data might be replaced with the income of another respondent of similar age, education, and gender. (See Brick, Kalton, & Kim, 2004, for more discussion.)

Numerous methods have been developed for imputation, ranging from the fairly simple and nonparametric hot deck to Bayesian model-based imputation (Little & Rubin, 2002). Multiple imputation is another frequently cited approach (Rubin 1987). As noted by Kalton and Kasprzyk (1986) and Brick and Kalton (1996), most of these methods fall within the general multiple regression framework.

Regardless of the method used, it is advantageous for a project like the NCS to produce filled-in, public-use data sets, as these can be analyzed by researchers without the need for sophisticated statistical modeling and missing-data adjustments.

Compensating for wave nonresponse in the NCS

Some NCS participants will fail to provide data for one or more of the survey waves. While some persons may drop out of the study at one wave and be lost for all subsequent waves (attritors), others may miss one wave but return to the study at a subsequent wave (nonattritors). The choice between weighting adjustments and imputation to handle wave nonresponse is not clear cut. In attrition nonresponse, however, weighting adjustments are usually preferred to a mass imputation of all variables for each of the missing waves.

Weighting adjustment for attrition nonresponse is relatively straightforward since this type of nonresponse is "monotone." Each successive wave adds an additional set of nonrespondents on top of those who dropped out in previous periods. Weighting adjustments at the current wave can be applied to the nonresponse-adjusted weights from the previous wave.

10.3.4.3 Compensating for Missing Data Under the Model-Based Approach

Missing data create a number of problems in statistical analysis. First, multivariate analysis methods typically assume complete data for all subjects. If some items are missing for a given subject, then the subject must be dropped from the analysis unless some form of adjustment is used (Vonesh & Chinchilli, 1997). A second issue concerns statistical efficiency since unavailability of some data elements decreases the effective sample size for statistical analyses resulting in wider confidence intervals and underpowered tests. A third issue is bias, since individuals with missing data may differ systematically from those with complete data. For example, in studies of health-related quality of life, subjects with the poorest quality of life are also most likely to be lost. Thus, analyses of the available data may be biased toward higher quality of life values.

However, a number of analysis methods can be used when missing data are present. The central tool for model-based analyses of longitudinal measurements is the linear mixed model (Diggle et al., 2002) as implemented in the SAS procedure Proc Mixed. The assumptions of MAR and parameter distinctness are sufficient to guarantee that likelihood-based analyses accomplished in Proc Mixed or similar software are correct even with substantial amounts of missing data.

Another analytic approach that avoids some of the stronger assumptions of likelihood-based analysis but still allows considerable flexibility in modeling is the generalized estimating equation (GEE) method (Liang & Zeger, 1986; Zeger & Liang, 1986; Diggle et al., 2002). With this so-called *marginal modeling* approach, one estimates a generalized linear model for the outcome variables (which can be either continuous or discrete), accounting for correlation within subjects (or larger units) by computing an adjusted variance matrix. Validity is robust to assumptions about the within-unit correlation. GEE modeling is valid under the assumption of MCAR though not generally under MAR. However, one can correct this by estimating a model for dropouts given observed data, and then weighting observations by the inverse dropout probability (Robins et al., 1995a, 1995b). A potential disadvantage is that GEE models describe only the marginal distributions of outcomes and therefore fail to capture within-subject correlation, which may be a critical feature of the phenomenon under study (Lindsey & Lambert, 1998).

Sensitivity analysis can be used to evaluate the impact of assumptions about the missingness mechanism. Pioneering work includes articles by Copas and Li (1997), Verbeke et al. (2001), and Troxel et al., (2004). A paper by Ma, Troxel, and Heitjan (2005) describes a method for local sensitivity analysis in a longitudinal model.

Another approach to handling missing observations is to impute them (as discussed in Section 10.3.4.3) and then analyze the filled-in data set as though it were complete. However, analyzing the filled-in data set without some accommodation for the imputation generally overstates precision. Rubin (1978, 1987) proposed multiple imputation (MI) as a way to avoid this difficulty. In MI analysis, one creates not one but several sets of filled-in data. The analyst then analyzes each filled-in data set separately, combining these results into a single overall analysis. Some MI algorithms are now available in commercial software, including the SAS procedure PROC MI.

10.3.5 Variance Estimation under the Design-Based Approach

The NCS is based on a complex sample design involving stratification and clustering by PSUs and by segments within PSUs. Under the design-based approach to inference, these features need to be taken into account in estimating the precision of estimates, whether they are basic descriptive statistics (means, percentages, totals in the total population or in subgroups) or analytic statistics (regression coefficients, odds ratios, etc). Failure to take account of the sample design in analysis can lead to invalid tests and erroneous conclusions (Skinner et al., 1989). This section briefly reviews the methods available for computing variance estimates for survey estimates based on complex sample designs.

Two approaches are commonly used for estimating sampling errors from complex sample designs: (1) Taylor series linearization methods, and (2) replication procedures. With the Taylor series method, estimates of variance are derived using a first-order Taylor series approximation of the deviations of estimates from their expected values. The required sums of squared deviations are then computed using the "ultimate cluster" approach described by Hansen, Hurwitz, and Madow (1953a, Chapter 6) and Kalton (1979). Replication methods, on the other hand, use subsamples of the full sample to obtain the standard errors of estimates (Rust & Rao, 1996). The subsamples, called "replicates," can take on a variety of forms including balanced repeated replicates, jackknife replicates, and bootstrap subsamples. A statistic of interest is calculated for the full sample and for each replicate, and the variability of the replicate estimates is used to estimate the variance of the statistic. An advantage of replication methods is they eliminate the need to specify complicated variance formulas (e.g., see McCarthy, 1966).

Both Taylor series and replication methods require appropriate variance stratum and variance unit codes in order to calculate the sampling errors. The variance units correspond to the actual first-stage sampling units within a stratum; thus, for the noncertainty PSUs, the variance units are the PSUs themselves, whereas within the certainty PSUs (which are, in reality, strata), the variance units are the sampled segments. (If a segment in a certainty PSU is also selected with certainty, the variance units would be the dwelling units.) Moreover, both methods require at least two variance units per variance stratum. In order to satisfy this condition, it may be necessary to collapse some of the noncertainty sampling strata for variance calculations. If collapsing is required, the resulting variances will tend to be overstated (Hansen, Hurwitz, & Madow, 1953b, page 218). To accommodate analyses that take the sample design into account, software such as WesVar, SUDAAN, and STATA can be used (LaVange et al., 1996; Research Triangle Institute, 2004; Westat, 2002).

10.4 Major Types of Analysis

10.4.1 Overview

This section discusses statistical methods that would be used as tools in the major types of analysis that will be undertaken in the NCS. The analyses are:

10.4.2 Cross-sectional exposure on an outcome;

10.4.3 Identifying causal pathways;

10.4.4 Analysis of neighborhood effects; and

10.4.5 Evaluating temporal effects.

Sections 10.4.2 through 10.4.5 discuss the specific statistical methods that will be used to achieve these analytical objectives. Before these methods are undertaken, however, certain basic, descriptive analyses will be carried out for all the variables being considered for inclusion in the planned analysis. For example, in the case of a dichotomous outcome, the prevalence of exposures (e.g., percent with detectable levels) from various sources (e.g., air, urine, blood, cord blood) will be compared between groups with and without the outcome. The distributions of quantitative exposures (continuous data) from these sources will be assessed with logarithmic or other transformations carried out as necessary to meet assumptions for statistical modeling. Finally, exposure levels measured from these sources will be characterized by the mean, standard deviation, median, and interquartile range. After these exploratory steps, detailed analysis will be done using the methods described in Sections 10.4.2 through 10.4.5.

10.4.2 Individual Cross-Sectional Exposure/Outcome Analysis

10.4.2.1 Linear and Nonlinear Regression

An important part of the data analysis in the NCS will be to investigate the association between an outcome of interest and some exposure measurement, controlling for possible confounders. For example, researchers may be interested in evaluating the association between prenatal exposure to polychlorinated biphenyls (PCBs) and cognitive and motor development in young children. Regression models, linear or nonlinear, are important analytical tools to address such scientific questions.

Linear regression methods are associated primarily with continuous outcomes. Many outcome measures in the NCS can be regarded as continuous in nature, including fetal growth, children's fine and gross motor skills, bone density, and heath care utilizations. Linear regression models can be used to study the relationships between such continuous outcomes and exposures of interest while controlling for confounders.

As an illustration, Daniels et al. (2003) examined the association between mother's prenatal exposure to PCBs and children's cognitive and motor development using data from the Collaborative Perinatal Project. The Bayley Scales of Infant Development were used to assess the infants' mental and psychomotor development at 8 months of age. PCB exposure represented the sum of 11 measured congeners from maternal nonfasting blood sampling collected at the third trimester. Possible confounders included maternal education, socioeconomic index, intelligence quotient, marital status, prenatal smoking status, prepregnancy body mass index, third trimester serum triglyceride, total cholesterol, and dichlorodiphenyldichloroethylene levels, the child's birth order, gestational age, and whether the child

ever breast fed. The investigators treated the Bayley Scales as continuous and fitted a linear regression on the exposure and the confounders.

On the other hand, many other outcomes in the NCS will be discrete. For example, congenital malformations can be categorized as present or absent. Outcomes may also take more than two levels of values. For example, a measure of activities of daily life may be coded as very good, good, bad, or very bad. Other outcome measures can be count variables, such as number of hospitalizations in a month. For these outcomes, nonlinear models are appropriate. In particular, logistic regressions (Hosmer & Lemeshow, 1989) are useful for modeling the association between a binary outcome and the exposure, controlling for the confounders. When the outcome is polytomous, some generalizations of the logistic regression can be applied. For example, when the levels of the outcome have no meaningful order, polytomous logistic models can be used (Hosmer & Lemeshow, 1989; McCullagh & Nelder, 1989). When the levels of the outcome follow a natural order, either the adjacent-category logistic model (Agresti, 1984), the proportional odds model (McCullagh & Nelder, 1989), or the continuation-ratio model may be used. When the outcome is a count measure, Poisson regression models or extensions of Poisson models are appropriate (McCullagh & Nelder, 1989).

It is likely that survival or time-to-event analysis will be appropriate for some outcomes, such as child development milestones or occurrence of childhood illnesses. The proportional hazards model is essentially a nonlinear regression model where the time to an event (e.g., infant's first steps or first holding a spoon) is the dependent variable. The potential effects of genetic and environmental factors can be modeled as predictors of delayed or accelerated development using the proportional hazards model (Marubini & Valsecchi, 1995). Variables that change over time (time dependent covariates) can also be included in the model. For example, environmental or other exposures that change with time can be included in the model.

Interaction terms can be added into both linear and nonlinear regression models when the exposure effect may vary with the level of a moderating variable. For example, if the decrease in motor development index for one unit increase in prenatal PCBs exposure is larger in breast-feeding children than in non-breast-feeding ones, then an interaction between the PCBs exposure term and the variable for breast-feeding can be added in the model. Variables such as gender, race/ethnicity, and age are likely to modify the effects of exposures in many NCS analyses.

The standard practice is to use forward or backward selection procedures in determining whether a confounder should enter the model. With these procedures, the main effects are selected first followed by the interaction terms. Likelihood or deviance measures are used to assess overall model fitting. Residuals and Pearson's residuals are used to address model diagnostics (Cook & Weisberg, 1982; McCullagh & Nelder, 1989).

10.4.2.2 Propensity Scoring

Propensity scoring provides an alternative method for controlling on confounder variables. Since its introduction by Rosenbaum and Rubin (1983; 1984), the method has become widely used for this purpose, particularly in biostatistical applications (D'Agostino, 1998).

With propensity scoring, exposure is viewed as a chance event where the chance of being exposed at a given level depends on the confounder variables. For example, breast-feeding rates differ substantially by race, socioeconomic level, and other demographic factors (Li & Grummer-Strawn, 2002). Thus, an infant's chance of being exposed to breast feeding depends on these factors, which may act as confounding variables in an analysis of true effects of breast feeding on healthy growth and development

outcomes. The first step of the analysis is to develop a model for predicting the exposure level given the confounders. In the simplest case of a dichotomous exposure (exposed vs. unexposed), a logistic model can be used. With several levels of exposure, an ordinal logistic model can be used (Joffe & Rosenbaum, 1999). An attractive feature of this approach is that a large number of potential confounders and interactions can be introduced into the models. An individual's propensity score for a particular exposure level is then given by his or her predicted probability of experiencing that exposure level.

Comparing groups at different exposure levels controlled on the same propensity scores removes the effects of the confounders included in the propensity model. Often, the propensity score distribution is divided into a number (5 to 10) of subclasses, and confounder control is carried out by performing the analysis within each subclass (see, for example, Rubin, 2007). The subclass results can then be combined in a weighted analysis. Tests of balance for each of the individual confounders can be carried out to check that the distributions of the confounders are equated across the exposure levels. Further balance can be achieved for key confounders by additional calibration procedures (see, for example, Judkins et al., 2006).

Propensity scoring bears a close resemblance to survey weighting. Survey weighting aims to achieve a weighted sample that mirrors the total population of inference. Propensity score weighting can be applied to make the weighted sample at each exposure level have the same standardized propensity score distribution. That standardized distribution could be the distribution for any one of the exposure levels or for the full population. The latter (standardized population) was used in an examination of the effect of the Youth Anti-Drug Media Campaign based on a National Survey of Parents and Youth (Orwin et al., 2006). The propensity score weighting was applied after the survey weighting, and it was reflected in the survey sampling variance estimation procedures used for testing the effects of different levels of exposure to the media campaign. Propensity scoring can similarly be applied in the NCS to examine the effects of environmental, socioeconomic, or other exposures on health and other outcomes, controlling for many potential confounders.

10.4.2.3 Exposure/Outcome Analysis

Overview

The NCS will collect data on many types of environmental exposures. In some cases, different exposures can have related human effects; in other cases, the same type of environmental exposure can produce multiple effects, each of which can be measured at multiple times during the course of the NCS. Thus, the analysis of relationships between exposures and health and other outcomes is necessarily complex.

For example, neurotoxins, such as lead, mercury, and persistent pesticides, and nonpersistent pesticides have similar health effects (Weiss, 2000). Ozone, allergens, endotoxins, indoor air contaminants, and mold affect asthma in similar ways (Gold, 2000; Sunyer, 2001). Potential endocrine disruptors, which appear to cause reproductive problems in birds and fish, can include insecticides, herbicides, industrial chemicals, and heavy metals (Landrigan, Garg, & Droller, 2003).

Some outcomes are accentuated by the interaction between exposures over and above the additive affects of the individual exposures. Across the large number of study participants and geographic locations in the NCS, many different combinations of exposures will be observed. The relative importance of each of the analytes and their interactions can be determined by including all the different analytes in a multivariate analysis. Pathways relating different exposures to each other, to mediators, and to an outcome can be analyzed using structural equation modeling. This method is especially useful for

creating a more comprehensive model of mediating mechanisms based on the results of univariate exposure/outcome analyses in the cohort. In this instance, structural equation modeling serves as a confirmatory method, assessing the fit or appropriateness of a proffered causal model rather than as a method used to develop a causal model.

The determination of the measure or measures of exposure to use in an exposure/outcome analyses will often be far from straightforward. Since exposure may derive from different sources at any point in time, it may be measured in several different ways and vary over time. Many of the exposures being measured in the NCS will arise in multiple media. For example, pesticides can be found in air, dust, soil, water, and food, and hormonally active agents can be found in drinking water, indoor air, food, soil, dust, and commercial products. Each of these exposures and media will be measured at multiple times during the course of the study. Exposure data can be collected with such measurement instruments as interviews, medical records, diaries, chemical environmental samples, biomarkers from blood or urine, and community level assessments. Measurement error in the various exposure measures also must be taken into account (see Section 10.3.3).

The sections that follow discuss, in turn, the analysis of exposure/outcome relationships that involve one exposure in multiple media with one outcome; multiple exposures with one outcome; and one exposure with multiple outcomes.

One outcome with multiple sources of exposure

In many cases, the NCS will collect data on exposures to contaminants that exist in multiple media or sources. The example given above is of pesticides, which can occur in air, dust, soil, water, and food. Since adverse outcomes may be accentuated by the interaction between multiple sources, it is important to include all the various sources in statistical models. Statistical models for exposure interaction will be similar to those discussed in the context of gene by environment interactions (Section 10.5.2), though environmental exposures are more likely to be continuously measured. For such models define:

E1 = exposure measure from source 1 or exposure type 1;

E2 = exposure measure from source 2 or exposure type 2;

Then the association with the outcome of interest may be modeled as,

$$\text{Logit}[\Pr(\text{outcome})] = b_0 + b_1 E1 + b_2 E2 + b_3 (E1 * E2).$$

As an example, consider "exposure" to phthalate esters. Phthalates have been shown to produce male reproductive tract malformations, including cryptorchidism or undescended testes (UDT), in rats when administered during sexual differentiation (Wilson et al., 2004). The quantification of phthalate levels in the human environment is crucial to determine whether exposures are sufficient to produce UDT or other "outcomes" in humans (Fisher, 2004). Phthalates are of particular concern because exposure is ongoing and ubiquitous (CDC, 2003; Silva, Barr, et al., 2004). They are widely used as softeners of plastics, solvents in perfumes, and additives to hairsprays, lubricants, and insect repellents. These exposures can be measured using air samples. Phthalates and its metabolized forms can be measured in biological samples such as maternal blood/urine, cord blood and infant urine, meconium, and amniotic fluid (Silva, Slakman, et al., 2004).

Swan et al. (2005) developed a global score for the assessment of the phthalates as a group that took into consideration exposures from multiple sources and diverse parent compounds. They constructed their global score by categorizing individual metabolite concentrations into low (below the 25th percentile), intermediate (between the 25th and 75th percentiles) and high (above the 75th percentile) groups, assigning a score to each group and then summing these scores across the metabolites measured in each urine sample. The use of a global score allows for assessment of the phthalates as a group and takes into consideration exposures from multiple sources and diverse parent compounds.

Once a subset of exposure summaries is constructed (e.g., cumulative exposure over time from different sources or peak exposure), correlations among the various summaries can be evaluated. This information along with estimates of the individual exposure associations with outcomes of interest can then be used to develop additional models. Latent variable models (Jöreskog & Sörbom, 1996) and generalizations of such models (Sammel & Ryan, 1996; Sammel, Ryan, & Legler, 1997; Muthén & Muthén, 2004) can be used to assess and validate proposed models of exposure and outcome relationships. (Latent variable models are discussed in more detail in Section 10.4.3.) These models should be used in a confirmatory setting for inferences regarding structure to be meaningful.

As an example, consider Figure 10-1, which illustrates how a latent variable for organophosphate (OP) exposure is derived from three observed sources of information. In this structural equations framework, the underlying, unobserved true OP exposure (represented by an oval) gives rise to the observed measured exposures (rectangles) from the various sources: two body compartment measurements and one indirect personal air monitor measurement. Each λ represents the contribution of a particular exposure type to a composite OP measure. The impact of "true" OP exposure on birth weight is estimated by θ. Statistical tests for θ are a type of global test for the impact of all the observed types of OP exposures on birth weight.

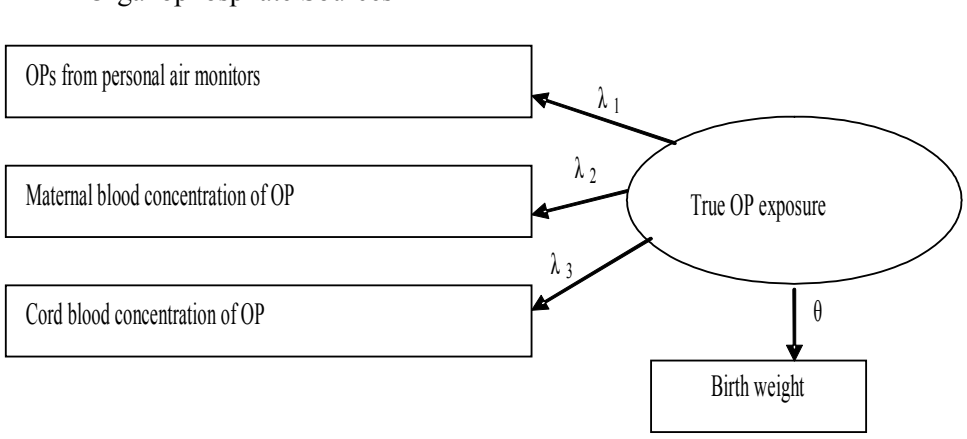


Figure 10-1. Path Diagram for Multiple Exposures

One outcome with multiple exposures

Another type of exposure/outcome relationship occurs when one outcome is associated with multiple exposures, which may act independently or in combination to influence the outcome. The issue of timing further complicates evaluating the effect of an exposure, since the time of exposure may significantly modify its influence on an outcome. Logistic regression can be used to estimate both the

independent and interactive effects of multiple exposures on the overall risk of a specific dichotomous outcome as well as the impact of timing of exposures.

Consider the example of preterm birth, which will be assessed in the NCS. Preterm birth is influenced by environmental, psychological, social, physical, and genetic factors. Two important mediators of preterm birth are inflammation and intrauterine growth restriction (Steer, 2006). The inflammatory response of the human body as it relates to preterm birth can result from bacterial vaginosis (Hartville, Hatch, & Zeng, 2005) and stress (Ruiz, Fullerton, & Dudley, 2003). In addition, stress can be the result of several factors, such as socioeconomic status (Misra, O'Campo, & Strobino, 2001) or lack of social support (Sheehan, 1998). Stress response itself is also mediated by endocrine function and corticotrophin-releasing hormone, which are related to the risk of preterm birth (Gennaro & Hennessey, 2003). The use of logistic regression modeling techniques will allow investigators to use NCS data to analyze how the interaction of these exposures affects the overall risk of preterm birth.

The impact of multiple interactive exposures on preterm births can be analyzed using a structural equation modeling framework (See Section 10.4.3). For example, Sheehan (1998) used structural equation models to show how economic stress, family stress, and lack of social support influence low birth weight.

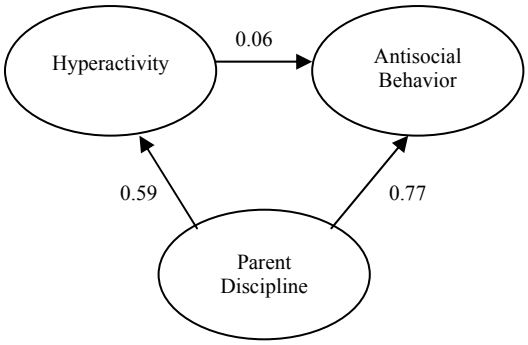
Multiple outcomes with a single exposure

Although some exposures lead predictably to a single identifiable outcome, both theory and empirical evidence suggest that a given exposure can lead to various, sometimes alternative, outcomes (Rutter, 1989). Elements of the environment, individual characteristics, and genetic predispositions can modify the trajectory such that the same exposure leads to a diversity of outcomes across, or even within, individuals. Both the various outcomes associated with an exposure and the moderating conditions that produce the different outcomes can be modeled using multivariate statistical techniques.

A set of trajectories with a unified starting point but multiple endpoints is described in the psychological and biological literature as multifinality (Cicchetti & Rogosch, 1996). Within the NCS, multifinality will arise with regard to processes of resilience. Resilience occurs when a child has an exposure that would logically lead to a negative outcome but does not due to a moderating influence. These individuals have outcomes substantially better than their history of exposure would predict, thus leading to a multifinality analysis. The resulting multiple endpoints consist of different and sometimes unrelated outcomes not simply different levels within the same outcome. For example, some children who experience physical abuse become highly aggressive toward others; some become vulnerable and open to subsequent exploitation; and others demonstrate remarkable resilience and exhibit high levels of behavioral and psychological competence.

Multifinality can be analyzed using one of several multivariate techniques that permit modeling of multiple, simultaneous dependent variables. One such technique is multivariate analysis of variance (MANOVA), which permits the prediction of multiple interval or ratio-level dependent variables from a common set of categorical independent variables. In the initial stages of analysis, MANOVA yields a multivariate *F*-statistic that indicates the significant effect of the independent variable across a multivariate response vector. Subsequent univariate tests are used to uncover the specific relations of the independent variable and any tested interactions with each of the dependent variables. The technique permits direct comparisons of strength of prediction to the multiple outcomes and the differential role of moderators across outcomes.

Multifinality can also be analyzed using structural equation models and path modeling where a single exposure can be modeled as resulting simultaneously in more than one outcome, and the relations between the exposure and each outcome can be compared. As an example, using structural equation modeling, Patterson, DeGarmo, and Knutson (2000) found that poor parental discipline predicted both child hyperactivity and child antisocial behavior in boys although the two outcomes were not significantly associated with each other (see Figure 10-2). The numbers on the arrows in the figure below are the estimated standardized coefficients for the indicated paths. Based on a separate analysis of the data, Patterson and colleagues suggested that parental antisocial behavior, a construct with a strong heritable component, might be the moderating factor that leads to this divergence in outcomes from parental discipline. The NCS would be well suited to test such multivariate models of multifinality.



Source: Patterson et al., 2000.

Figure 10-2. Model of Multifinality of Parent Discipline

10.4.3 Identifying Causal Pathways

The Children's Health Act of 2000, which authorized the planning and implementation of the NCS, also directed the Study to "investigate basic mechanisms of developmental disorders and environmental factors, both risk and protective, that influence health and developmental processes." This means that, in addition to establishing cause/effect relationships, the NCS has been directed to investigate the mechanisms mediating these associations. As an example, a number of factors have been shown to be associated with preterm birth: infection/inflammation, environmental toxins, and behavioral/psychosocial factors. What are the mechanisms through which these factors influence prematurity? Do they involve separate, independent pathways? Do they cumulatively influence the same pathway, such as prostaglandin synthesis, or do they interact in some other way? The objective of the NCS is not only to establish which risk or protective factors are associated with which outcomes but also to increase our understanding of how this occurs so that preventive efforts can be more specifically focused.

Structural equation modeling (SEM) can be used to address such issues. This method allows significant pairwise associations found between a set of single factors to be placed in a larger, theoretically derived contextual model with other significant associations to examine the interrelationships.

Thus, SEM can be used to examine relationships between exposures, mediators, and outcomes in a single overall model. The technique combines multiple regression, path analysis, and factor analysis to assist in causal inference. SEM extends the general linear model since it estimates simultaneous relationships between multiple covariates and responses (outcomes), possibly including unknown latent variables.

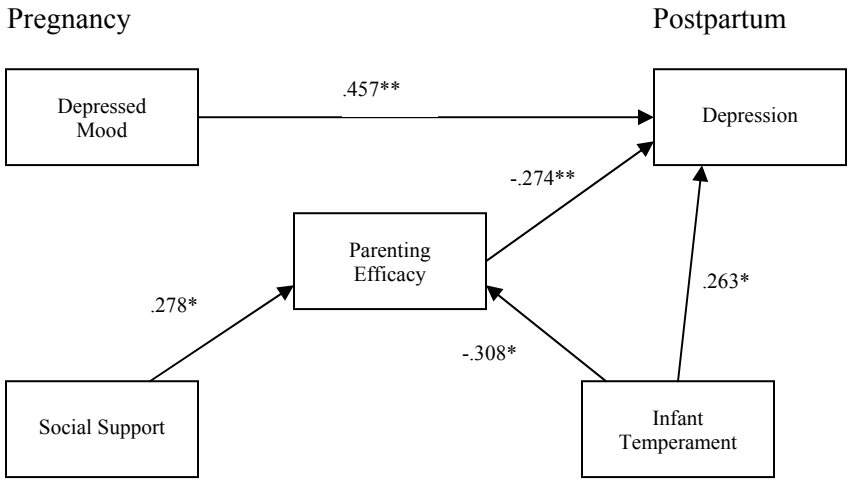
A major strength of SEM is its ability to also model constructs as latent variables simultaneously by means of separate regression equations (Bollen, 1989). Latent variables are unobserved variables or constructs (e.g., IQ) estimated indirectly in the model using measured variables (indicators) that affect them. They can be used as either independent or dependent variables.

SEM/LISREL[1] modeling focuses on two steps (Jöreskog & Sörbom, 1996): validating the measurement model and fitting the structural model. The measurement model specifies how latent variables/hypothetical constructs depend upon the observed variables, and how the association between observed variables is mediated through other observed variables. The structural model specifies the causal relationships between latent and/or observed variables, describes the causal effects, and assigns the explained and unexplained variance. At the outset, one specifies a model based on the underlying theory.

Some of the analyses in the NCS will involve SEM models that do not include latent variables. SEM also works very well in these cases (Bollen, 1989) where the objective is often to understand mechanisms that mediate the association between multiple exposures and a single outcome. As noted earlier, many of the conditions (e.g., premature birth) that the NCS plans to investigate will show associations with multiple psychosocial and physiological factors, and the aim will be to explain the causal mechanisms. For example, if infection and inflammation are shown to explain a significant amount of the variance in premature births and this is also the case with environmental toxins and stress, what are the causal pathways and how are they mediated?

A study of maternal postpartum depression by Cutrona and Troutman (1986) provides an illustration of this type of analysis. The study sought to identify maternal and child qualities associated with changes in depression from pregnancy to the postpartum period. The model was based on theoretically predicted relations from previous research on bivariate relations and significant associations resulting from subsets of analyses where social support, infant difficult temperament, and parenting efficacy were related. Thus, the model was built on theory and preliminary data analyses. Combining these multiple variables into a single model demonstrates, for example, that while having a temperamentally difficult infant predicts greater increases in depression directly, part of this effect is also mediated through feelings of efficacy about parenting (see Figure 10-3 below). Such analytic models inform both more in-depth research questions and process avenues for intervention.

[1] The terms SEM and Linear Structural relationships (LISREL) will be used interchangeably from here onwards.

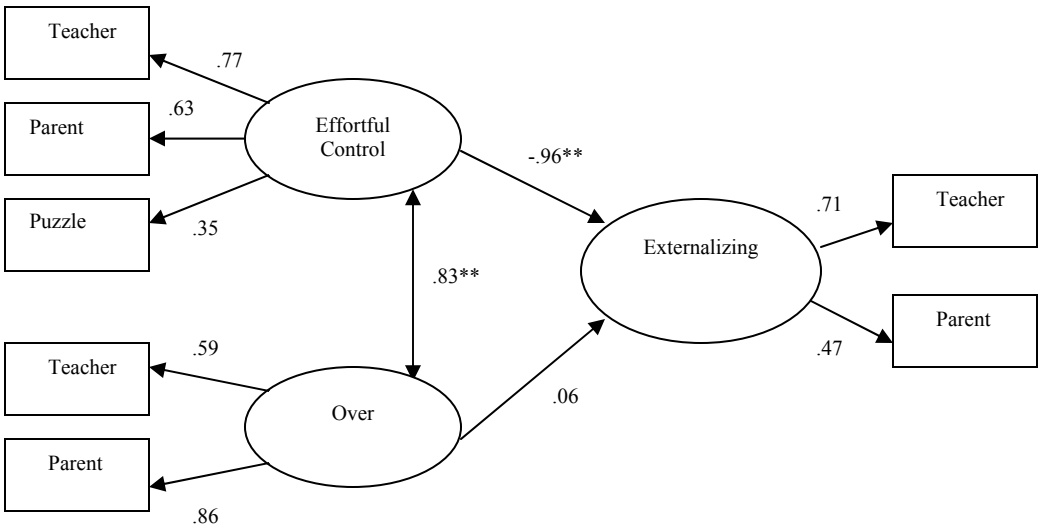


* significant path, p < .05
** significant path, p < .01
Source: Cutrona & Troutman, 1986.

Figure 10-3. Social Support, Infant Temperament, and Parenting Self-Efficacy as Predictors of Postpartum Depression

Other analyses in the NCS will involve multiple observed indicators of latent constructs, and, consequently, latent variable models will be applicable. The latent variable model of child temperamental self-control and aggressive behavior problems developed by Valiente et al. (2003) provides an example (see Figure 10-4). The latent independent variables in their model are temperamental effortful control and temperamental over-control (ellipses in the figure below). The observed variables (rectangles) associated with effortful control are parent, teacher, and task observation ratings of relevant child attention skills and persistent behavior. The observed variables (rectangles) associated with over-control are parent and teacher reports of relevant over -controlled behavior. The latent dependent variable is behavior problems (ellipse). The observed indicator variables are parent and teacher reports of child aggressive behavior. The arrows for the latent variables point toward the observed variables associated with them. The numbers on these arrows are the estimated standardized regression coefficients for the structural equation and measurement models.

This model indicates a direct relation between effortful control and child externalizing behavior. No significant direct relation is found between over-control and externalizing behavior with effortful control simultaneously accounted for in the model. The use of latent variables in this model permits more robust measurement of the latent constructs than would an analysis including only one observed assessment of each construct from a single reporter.



** significant path, p < .01
Source: Valiente et al., 2003.

Figure 10-4. The Relation Between Child Self-Control and Aggressive Behavior Problems

10.4.4 Analysis of Neighborhood Effects

The design of the NCS involves clustered sampling with clustering at the county level, and, within the county, at segment level. This type of design has the analytic benefit of providing a structured set of geographically defined neighborhoods for evaluating the effects of exposures that occur at the neighborhood level. Neighborhoods may be defined in a number of ways depending on the type of exposure and hypothesis being tested. For example, school districts might define a neighborhood for school performance assessments while geographic or administrative boundaries might define a neighborhood for examination of environmental exposures related to the water supply.

Data arising from neighborhoods or clustered structures have a hierarchical form since individual-level data can be grouped within a higher category. Since hierarchical data are nested within a higher structure, there is generally some degree of correlation between observations. For example, two individuals within one community generally are slightly more similar with regard to such factors as religiosity, socioeconomic status, education, and environmental exposures than two individuals sampled from different communities. Multilevel modeling (MLM) is the primary analytic method for analyzing the effects of the hierarchical structure.

MLM is also known as the random coefficient model (Rosenberg, 1973) and as the hierarchical linear model (HLM) (see Bryk & Raudenbush, 1992). MLM is based on the mixed-effects model with both fixed and random components. The regression coefficients are treated as random variables that can vary depending on the higher level unit (e.g., neighborhood). Consequently, MLM can be used to develop regression models with intercepts and regression weights across higher level units as outcomes and other higher-level variables as covariates.

Hierarchical models play an increasingly important role in epidemiology. For example, Juhn et al. (2005) assess the influence of neighborhood and individual-level factors on the incidence of childhood asthma among children born in Rochester, MN, between 1976 and 1979. The neighborhood-level variables considered in this study include collective efficacy, social cohesion, neighborhood socioeconomic status, and whether the Census tract contains major highways or railroads.

An important application of hierarchical modeling with NCS data will be to examine neighborhood effects on various health and developmental outcomes. The collection of standardized information on neighborhoods and counties will permit analysis of effects of both neighborhood-level and individual-level factors.

A hierarchical model can be fitted using either Monte Carlo methods (Gelfand & Smith, 1990) or some approximate methods such as penalized likelihood, penalized quasi-likelihood (Breslow & Clayton, 1993), and restricted iterative generalized least squares (Goldstein, 1995). Interaction terms can be added in the same way as in linear and nonlinear regression models. There are several software programs available for fitting multilevel models, including MLwiN (www.cmm.bristol.ac.uk/), HLM (www.ssicentral.com/), and WinBUGS (http://www.mrc-bsu.cam.ac.uk/bugs/).

10.4.5 Evaluating Temporal Effects

10.4.5.1 Overview

The longitudinal design of the NCS has the important advantage of permitting the evaluation of temporal effects. There are several statistical tools available for evaluating temporal effects depending on the research question being asked and the type of data being collected. This section discusses longitudinal data analysis, structural equation modeling with longitudinal data, and growth curve models.

10.4.5.2 Longitudinal Data Analysis

Longitudinal data analysis most often refers to repeated measurements on individuals. In the NCS, data will be collected from individuals at regular time points throughout the period of study participation. Specifically, physical, cognitive, and intellectual growth and functioning will be measured over many years. Because these measurements are taken from the same individuals over time, they are correlated and, thus, require special tools for data analysis.

The primary methods used in longitudinal data analysis are generalized estimating equations (GEE) and mixed effects models (see, for example, Fitzmaurice, Laird, & Ware 2004). Since repeated measures may be considered to be "nested" within individuals, multilevel models can be used to reflect the dependent structure of the observations (see Section 10.4.4).

In mixed effects models, individual effects are modeled explicitly. In essence, a separate regression equation is estimated for each individual based on the values of the dependent variable and the independent variables measured at the different time points. The repeated measurements are assumed to be independent within a given subject after the individual intercept and slope for the subject is taken into account. For example, in modeling dental growth, an intercept and slope might be fit for each individual; variations in projected growth about the regression line for an individual are assumed to be independent. In this example, a mixed model would include random effects for growth variations in each individual and fixed effects for factors such as gender that affect overall growth rates.

GEE is associated with marginal models, so-called because they are based on data that are averaged or accumulated for each time point. That is, the basic model is $E(\boldsymbol{Y}_i) = X_i'\beta$, where $\boldsymbol{Y}_i$ is a response vector at time i, X_i is an independent variable measured at time i, and β is a fixed regression coefficient. For example, Diggle, Liang, and Zeger (1994) show how marginal models using GEE can be used to compare respiratory infection rates between children with and without vitamin A deficiency using examination data from six medical visits, adjusting for the effects of seasonality and age**.** While marginal models and mixed effect models give similar results for continuous outcomes, GEE is more suited for binary outcomes.

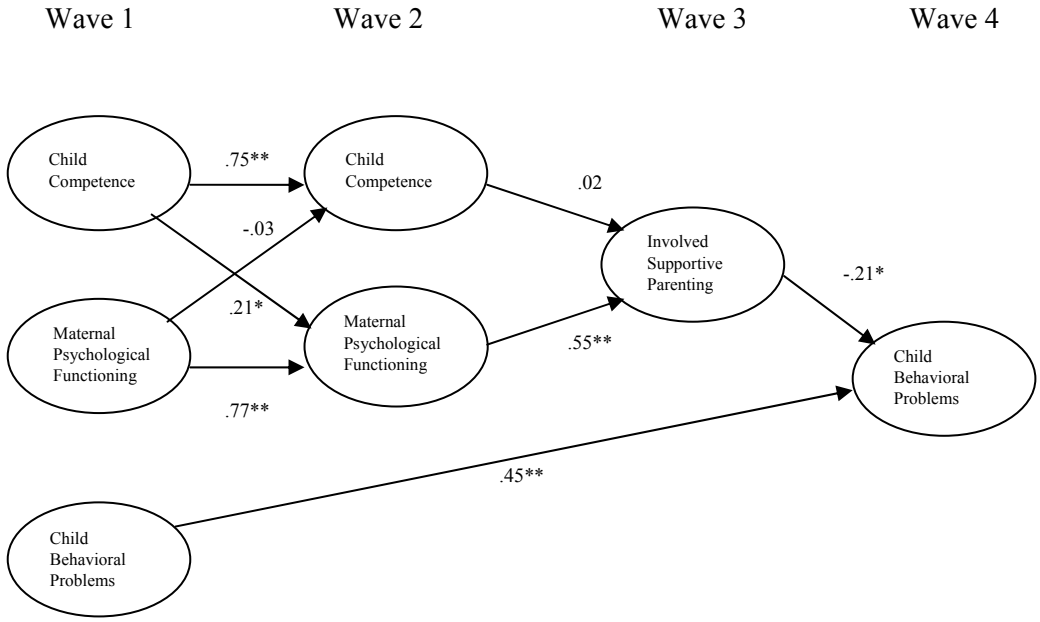
10.4.5.3 Longitudinal Structural Equation Modeling

Structural equation modeling, described in Section 10.4.3, has particular utility for modeling longitudinal data. Data sets such as the NCS that include multiple repeated measures of constructs or indicators are well suited for this analytic strategy. SEM permits modeling of latent variables from multiple indicators and the simultaneous testing of the interrelations among latent variables or individual observed variables. Longitudinal SEM extends this to make multivariate modeling of change over time possible.

When repeated measures over time are represented in SEM models, it is possible to examine predictions of later values of a latent construct while simultaneously accounting for stability of the construct from a previous assessment. Consequently, the predicted outcome represents change in that variable from the previous time point rather than being a static assessment. Because it permits complex multivariate analyses through robust model estimation techniques, SEM provides one of the most statistically elegant methods for investigating stability and change in longitudinal research.

Longitudinal SEM models also permit highly complex, temporally accurate testing of latent variable mediation models, such that the exposures precede the mediator and the outcome follows the mediator temporally, reducing the possibility of reverse-mediation. When previous values of the variables are also accounted for in the model, longitudinal SEM mediation models provide greater support for the hypothesized causal pathways than do models without these multifaceted longitudinal components.

An example of SEM with four waves of data concerning maternal and child adjustment is presented by Brody, Kim, Murry, and Brown (2004). Within these four waves were latent variables at two time points each for maternal psychological functioning, child competence, and child behavioral problems as well as a latent variable for involved supportive parenting (see Figure 10-5 below).



* significant path, p < .05
** significant path, p < .01
Source: Brody et al., 2004.

Figure 10-5. Longitudinal SEM Model of Child and Maternal Functioning

The longitudinal nature of the data permits complex inferences about the interrelations of these constructs over time. As can be seen from the figure, Wave 1 child competence predicts changes in maternal psychological functioning between Waves 1 and 2, which subsequently predicts involved supportive parenting at Wave 3. The figure suggests that child competence has effects on later parenting behavior only through its effects on intervening maternal psychological functioning. Involved Supportive Parenting also predicts changes in child behavior problems between Waves 1 and 4. Longitudinal SEM effectively models the complex bidirectionality of influence that parents and children have on each other's functioning over time. The NCS data will permit modeling such as this over the course of many waves of data and for multiple sources of influence.

10.4.5.4 Growth Curve Analysis

The primary goal of growth curve analysis is to describe patterns of change over time and identify predictors that affect these patterns. Growth curves may be modeled using exponential or logistic functions when early growth is rapid. Polynomial growth curves may also be useful. As a familiar example, height has a well understood developmental trajectory where a logistic growth curve fits the first three years of life, followed by a linear growth trend until adolescence when growth again follows a logistic curve (Bock et al., 1973).

Another example of growth curve analysis is given by Cherlin et al. (1998), who describe the effects of parental divorce on the subsequent mental health of their children at ages 7 through 33. This study, which was based on data from the National Child Development Study (Chase-Lansdale et al., 1995), assigned scale scores to emotional problems at a range of ages. Growth in these scales was then modeled as a function of age at time of divorce, gender, economic status, class background, and school

achievement. All variables except class background were statistically significant. The significant age at time of divorce variable resulted from higher scores on emotional problems scales for later ages at time of divorce up to age 22.

The latent growth curve model (LGM) is a special case of structural equation modeling and multilevel modeling. LGM treats repeated measures of individual behavior as a function of chronological development. LGM is a type of multilevel model that extends the hierarchical structure to panel data in which individuals are observed across time.

10.4.6 Case-Control Studies

10.4.6.1 Introduction

In case-control studies, a small number of cases (usually persons with a particular disease or other outcome of interest) are compared with a sample of controls, persons without the disease or the outcome being studied. There are several potential situations where this method would be relevant to the NCS. One example would be where blood or other specimens have been stored for many NCS participants but, due to laboratory processing expenses, only a relatively small number of individuals (with a given condition) would actually be selected to have their specimens analyzed. In such a situation, the specimens of an appropriate set of controls (persons without the condition) would also be analyzed and the two sets of data would then be subjected to a case-control analysis. A similar situation might arise if a new hypothesis called for additional measurements to be taken.

A second situation, which is the more traditional application, would arise when individuals with a given outcome are rare. In such a situation, all persons sampled in the NCS with the condition might be included in a comparison with a selected sample of controls to evaluate risk factors, for example.

10.4.6.2 Selection of Controls

The selection of controls is critical to the validity of case-control studies. Quite often, some form of matching is used in this process to control for confounding variables known to influence disease incidence or outcome. The two basic methods of matching are set matching, where each case is matched individually to one or more controls, and frequency matching, where cases and controls are matched in categories (e.g., a group of 15 cases who are male and aged 30 to 39 might be frequency matched to 45 controls who are male and aged 30 to 39). While set matching is a more traditional approach, frequency matching has a number of advantages both operationally and analytically, particularly in large studies.

Because case-control studies typically begin with disease cases that have already occurred, they are subject to significant sources of bias. A key step in the process of ensuring a bias-free case-control study is that cases be representative of all those who develop the disease under investigation. One threat to this process is that cases are often identified as they are diagnosed in a clinical setting, and mild cases or those that result in early mortality will not be diagnosed and are thus missed as cases. This type of bias is called incidence-prevalence bias or survival bias. Case-control studies can also give biased results if the controls are not representative of the population at risk for developing the disease under investigation. To avoid these types of bias, it will be of paramount importance for the NCS to select appropriate and representative cases and controls.

10.4.6.3 Case-Control Studies in the NCS

Nested case-control studies are those where cases are identified within a well-defined cohort, such as the NCS, where controls are selected from within the same cohort. Nested case-control studies combine some of the advantages of both cohort and case-control designs. Depending on how control subjects are selected, a few sources of bias inherent to the case-control design (e.g., recall of events and chronological differences between case and control identification) can be avoided using a nested case-control investigation. Extensive data representing the time period prior to the disease diagnosis will be available for cases, and corresponding data will be available for controls. Another weakness inherent to case-control studies that will be prevented by selecting cases and controls from the NCS cohort is the lack of extensive records before disease diagnosis. Data on many key exposures not typically available in case-control studies, such as dietary patterns, medication use, and environmental exposures, will be available to NCS researchers to determine pre-morbid risk factors more accurately. The NCS is also expected to utilize the matched case-control study design, where individual cases are matched to one or more controls based on similar demographic characteristics suspected of confounding the relationship between the exposure and outcome association under investigation.

10.4.6.4 Analysis of Nested Case-Control Studies

When analyzing nested case-control studies, the use of standard survey weights will generally lead to unacceptably large variability in estimates. By design, controls are matched to cases in terms of key confounding variables, and the distribution of these variables in the cases is usually very different from the distribution in the general population. This feature gives rise to large variation in sampling weights, which, in turn, leads to large standard errors. There are several ways to avoid this problem, two of which we describe below.

One approach is to perform an unweighted analysis. This type of analysis of case-control studies provides estimates of relative risk but not absolute risk. Thus, for example, estimates of regression coefficients would be useful but not of the intercept. Alternatively, if weights are to be used, a possible approach is to weight the sample to the case distribution, thus compensating for any disproportionate sampling of cases or nonresponse. See Scott (2006) for a discussion of these issues.

In case-control studies that used set matching, the multivariate analysis technique most frequently used is conditional logistic regression. This estimation takes into account the pairing or matching of cases and controls with respect to the variables that determined the matching. The interpretation of the coefficients in conditional logistic regression is the same as in ordinary logistic regression except that these coefficients are to be considered “adjusted” not only for the variables included in the model but also for the matching variables.

For the statistical analysis of case-control studies using frequency matching, a more efficient strategy is to use ordinary logistic regression and include the matching variables in the model. Similarly, nested case-control studies can be analyzed in the same way as matched case-control studies, where cases and controls are matched by length of follow-up. As a result, the multivariate analysis technique most often employed is conditional logistic regression, in which the conditional variable is length of follow-up. This type of conditional logistic regression model is similar to the Cox proportional hazards regression model.

10.5 Analysis of Genomic Data

The combination of a longitudinal follow-up of the NCS cohort, its large size, and its comprehensive collection of environmental exposures will provide a rich source of data with which to investigate the contribution of genetic variation to complex diseases such as autism, obesity, and asthma as well as the impact of gene and environment interactions on neurodevelopment, health, and behavior outcomes. For either genome-wide analysis (GWA) or candidate-gene approaches, the outcome can be discrete (e.g., having autism or not), continuous (e.g., quantitative measurements of depression) or censored survival data (e.g., time to onset of type 1 diabetes).

With respect to complex phenotypes that will be studied in the NCS, it is expected that multiple genes as well as gene-environment and gene-gene interactions play a key role. The large sample sizes available will greatly facilitate not only the identification of individual genes, but also the ability to identify gene-gene and gene-environment interactions. It is nevertheless clear that the ultimate success of such GWAs will depend largely on the development of highly complex and innovative analytic strategies. Methods that can efficiently account for the genome-wide linkage disequilibrium patterns and control for genome-wide error rates using false discovery rate procedures are required. Novel statistical methods that can identify gene-gene and gene-environment interactions and methods that can incorporate known biological knowledge, such as networks and pathways, in searching for complex disease genes are also greatly needed. The analysis of genomic data is a field of much active research. Analysis of genotype effects and multilocus genotype-by-genotype interactions (e.g., epistasis) as well as gene-environment interactions can be cast in a regression framework for different types of outcomes where the predictor variables include SNPs, environmental exposures, interactions among SNPs, and SNP-by-environment interactions. Due to the problem of high-dimensionality, standard regression analysis methods cannot be applied directly when many genes are involved because they produce highly variable estimates. Methods developed for analyzing high-dimensional data, such as microarray gene expression, massively parallel signature sequencing (MPSS), and evolutionary trees of haplotypes, may also be utilized. New analytic methods can be expected to emerge in the future, and researchers analyzing the genomic data in the NCS will need to apply the best methods available in every phase of the process. Some specifics of these methods are described below.

10.5.1 Haplotype Analysis

Recent advances in high-throughput technologies and the decrease in genotyping costs have made genome-wide association analysis a feasible tool in the search for genetic contributors of complex traits, including many complex diseases. As the NCS evolves and technologies mature, it is possible that genome-wide genetic profiling for each participant of the study will be available to enable possible genome-wide searches for genetic variants as well as the interactions among genes and between genes and environmental risk factors. One challenge of such data is their very high dimensionality. One solution to this problem is to fully utilize the information from tagging SNPs, haplotypes, and haplotype blocks derived from the HapMap project. Haplotypes are a set of closely linked genetic markers present on one chromosome which tend to be inherited together and which can be utilized as the unit of analyses in order to examine their effects and interactions with the environmental exposures. Haplotype analyses are potentially more powerful in identifying genes predisposing to certain health outcomes. For example, a test for association between the common haplotypes in haplotype blocks and specific outcomes can be conducted. Such association analysis can be performed using sliding windows of a small number of overlapping SNPs. Alternatively, newly developed methods such as Logic regression, FlexTree, and threshold gradient descent procedures can also be applied for considering haplotypes in multiple regions and for identifying haplotype-by-haplotype interactions and haplotype and environmental exposure interactions.

One difficulty with haplotype analysis is that haplotypes are often not observed but must be estimated from the genotyping data. This can be accomplished in a regression analysis and missing data context. The expectation-maximization (EM) algorithm can be developed for estimating the model parameters (Lin, 2004). Such EM-based estimation as well as inference procedures have been developed for binary outcomes in case-control designs and for survival outcomes in prospective cohort designs. Following the nonparametric maximum likelihood approach in Scheike and Juul (2004) for estimation of the Cox model under nested case-control sampling, methods for analyzing age of onset data and for estimating the haplotype effects could be developed in a framework of censored data regression and missing data under the case-control and nested case-cohort samplings. Specifically, we can treat the haplotype phases and the haplotypes of those who are not genotyped as missing data and use the EM algorithm and the nonparametric maximum likelihood approach to estimate the haplotype relative risk and the baseline hazard function (Chen & Li, 2005, in preparation).

10.5.2 Population Stratification

Population stratification is an important issue to consider when studying gene-trait associations using unrelated subjects, since the observed association could be spurious without appropriate adjustment for underlying population strata (Cardon & Palmer, 2003). In our analyses, we will often consider African Americans separately from Caucasians. However, population substrata could still confound gene-trait associations within African Americans and Caucasians, particularly in African Americans (Cardon & Palmer, 2003). Therefore, it will be important to adjust for population stratification in genetic association analyses. If candidate gene studies are conducted, we will select ancestry informative markers in both ethnic groups and use the STRUCTURE (Pritchard & Rosenberg 1999) approach to infer the degree of population stratification as represented by the proportion of ancestry of each individual in the study. We will test the hypothesis of two or more strata (i.e., ethnic subpopulations) within each ethnic group, and the STRUCTURE program will attempt to classify individuals as belonging to one population or another. If there is evidence of population stratification, then the multivariate analyses described previously will be repeated with adjustment for population stratum membership (e.g., using stratified logistic regression analysis). For genome-wide association studies, a recent publication by Price et al. (2006) proposed a method that enables detection and correction of population stratification on a genome-wide scale using the idea of principal components analysis. The resulting correction is specific to a candidate marker's variation in frequency across ancestral populations. We will use this method and the EIGENSTRAT software provided by the authors for genome-wide association analysis.

10.5.3 Gene-By-Gene/Gene-By-Environment Interactions

Gene-environment interactions are measured by the effects of clinical/environmental exposures on the disease risk among individuals with different genotypes. Gene classification schemes can be added to the final model for clinical risk factors, and then systematic tests for interactions between gene classification and risk factors can be conducted. Logistic regression models can be utilized to explore three types of interactions. For notation, we define:

R = clinical risk factor(s);

E = exposure(s);

G1 = genotype/haplotype/single nucleotide polymorphism (SNP) 1;

G2 = genotype/haplotype /SNP 2.

Then,

Model 1: Clinical risk factor and gene interaction

$$\text{Logit}[\Pr(\text{outcome})] = b_0 + b_1 R + b_2 G1 + b_3 (R * G1)$$

Model 2: Environmental exposure and gene interaction

$$\text{Logit}[\Pr(\text{outcome})] = b_0 + b_1 E + b_2 G1 + b_3 (E * G1)$$

Model 3: Gene–Gene interaction

$$\text{Logit}[\Pr(\text{outcome})] = b_0 + b_1 G1 + b_2 G2 + b_3 (G1 * G2)$$

In the first model, the outcome variable, say a diagnosis of undescended testes (UDT) would be an indicator of status (0 = absent or 1 = present). A previously selected exposure of interest (e.g., phthalates) would be categorized as absent or present (E = 0 or 1 depending on whether the subject was exposed during gestation) or as a continuous variable (quantitative measure of phthalate exposure from biologic specimens or from an estimated latent variable), and a candidate genotype/haplotype (e.g., HOXA9) would be characterized as nonsusceptible/susceptible (G1 or G2 = 0, 1).

Estimation of the odds ratio for clinical risk factors among the susceptible genotype (G1 = 1) group is then $\exp(b_1 + b_3)$ and for the nonsusceptible group (G1 = 0) is $\exp(b_1)$. A measure of the strength of the interaction can be evaluated by the odds ratio (OR) for susceptible and nonsusceptible, which is expressed in this model as $\exp(b_3)$. A score test for the statistical significance of the interaction OR = 1 is then a test of $b_3 = 0$. The same approach would be taken to model phthalate exposure and gene interactions (model 2), and gene-gene interactions (model 3) (Hwang et al., 1995; Yang & Khoury, 1997; Andrieu & Goldstein, 1998) such as INSL3 and GREAT, both thought to control development of the gubernaculum.

Completion of the statistical analyses described here would allow not only for the assessment of associations between allelic variants in candidate genes but also for interactions between clinical factors and in utero environmental factors such as exposure to phthalates on the risk of the outcome of interest (e.g., nonsyndromic UDT).

Another area of study is the relationship between the environmental exposures and the patterns of somatic mutations in genes. To account for potential dependency of the mutation patterns along the genome, the generalized estimating equation approach for analyzing correlated binary data can be applied to identify how environmental exposures can potentially induce somatic mutations in cells. Clustering analysis methods can also be applied to cluster the mutation patterns based on multivariate binary data and to relate the mutation clusters to environmental exposures.

10.5.4 Regression Tree Approaches to the Analysis of Interactions Between Genes

Because interactions play a key role in the analysis of genomic data, including data involving SNPs, there are multiple approaches to this type of analysis. Since the number of nucleotides

involved in susceptibility for a complex traits and conditions is often quite large, special methods are required for analyzing the even larger number of possible interactions of SNPs within and between genes. As an example, the risk of type 1 diabetes can be related to the interaction of multiple SNPs rather than to single variation sites. Analytic approaches such as the adaptive spline and tree-based methods such as MARS and CART (Friedman, 1991; Breiman et al., 1984) can be used to generate interpretable interaction rules among the SNPs. Realizing the limitations of these methods—for example, MARS is efficient on data that has interactions in at most a few variables, and CART only generates rules in disjunctive normal form—the recently developed adaptive regression method, logic regression, may be applied in order to construct predictors as Boolean combinations of the SNPs using simulated annealing.

Such Boolean combinations of the SNPs may not be detected by a standard regression tree as implemented in CART. In order to study and assess gene-by-gene interaction and gene-by-exposure interactions on the risk of developing certain outcomes, the recently developed tree-based method FlexTree (Huang et al., 2004) may be employed. FlexTree is an extension of the binary tree-structured approach such as CART and is particularly applicable to study gene-by-gene and gene-by-environment interactions. The methods work well for both the model where many genes are involved in the predisposition of certain outcomes and the model where only a small list of aberrant genotypes is predisposing.

The Bayesian variable selection approach introduces a latent binary vector to index all possible subsets of variables (George & McCulloch, 1993). A prior distribution is specified for this latent vector and the variable selection is performed based on the posterior model probabilities. When the number p of covariates is large, deriving the posterior probabilities of all 2^p possible models is computationally prohibitive. This can be handled via Markov Chain Monte Carlo (MCMC) stochastic search techniques, which are used to explore the space of variable subsets and search for promising models. At each MCMC iteration, a new candidate model is visited and retained based on its posterior probability relative to the previously visited model. This method is well suited for the analysis of high-dimensional data where the sample size is substantially smaller than the number of covariates $(n << p)$. Another advantage of the Bayesian approach is that it allows the uncertainty inherent in the model selection process to be incorporated in the inference mechanism. This is accomplished via model averaging where the estimation of parameters and the prediction of future outcomes are computed by averaging over a range of likely models.

10.5.4.1 Multifactor Dimensionality Reduction for the Analysis of Interactions Between Genes and Between Genes and the Environment

Multifactor dimensionality reduction is a method designed specifically for investigating multiple gene-gene and gene-environment interactions. In studies using related family members, the programs are currently only applicable for gene-gene but not gene-environment interactions. However, most of the children in the NCS will be unrelated and therefore this method will also be ideal for the investigation of gene-environment interactions. In logistic regression models, the number of possible interaction terms grows exponentially as each additional main effect is added (Ritchie et al., 2003). To reduce the dimensionality in data where interactions are the primary focus, one divides the data into training and test sets and then forms all possible permutations of the chosen interaction terms. The interactions become, in a sense, the "main effects" under investigation (Ritchie et al, 2003). An example would be two genes, each with a dominant and recessive homozygote and a heterozygote, making nine possible combinations. Each cell class is labeled as high or low risk according to a predefined threshold. The MDR model that has the fewest misclassified individuals is selected, and the process is then repeated using 10-fold cross-validation to evaluate predictive ability and reduce spurious significances. Since

multiple gene-gene and gene-environment interactions are the rule rather than the exception in many complex health conditions and behaviors being studied in the NCS, this process will allow for more realistic modeling of multiple interactions.

10.5.4.2 Complementary Modeling Approaches

Interactions confirmed with any of the above-mentioned approaches can also be incorporated with other causative factors into path analytic models to identify multiple cross-sectional and/or longitudinal causal pathways (see more descriptive discussions in Section 10.4). An example would be the NCS hypothesis that gene-environment interactions between ozone exposure and polymorphisims of TLR-4 and/or TNF-α play a causal role in asthma onset. This could be tested with one of the methods described above and the significant interactions inserted into a path analysis model with other factors related to the outcome but not to these interactions (e.g., prenatal factors such as low birth rate).

10.5.5 Gene Expression Data-Microarray Analysis

In addition to these established methods, others developed for analyzing high-dimensional data such as microarray gene expression data are worth exploring. Gene microarrays are a method employed for examining the expression of as many as hundreds or thousands of genes in a single tissue (Jarvis, 2006). Although the NCS will not have tissue specimens, it will have whole blood. As the Study evolves, it may be possible to collect the gene expression profiles in whole blood samples over time in order to examine how those gene expression changes detectable in blood are related to development of various health outcomes or to identify potential biomarkers for diseases and investigate how gene expression is affected by environmental exposures. Such data make it possible to learn how expression of different genes and, hence, their coded proteins, interact to provide insight into biochemical pathways and causal mechanisms on a genomic level.

Of particular interest with respect to analysis of these data are the threshold gradient methods (Friedman & Popescu, 2004; Gui & Li, 2005) and the Bayesian variable selection methods (Sha et al., 2004) for identifying important SNPs, environmental exposures, and their interactions for the risk of developing certain health-related outcomes. Bayesian variable selection methods have been developed and used successfully for the analysis of DNA microarray data in the context of multigroup classification (Sha et al., 2004) and clustering (Tadesse, Sha, & Vannucci, 2005). In the former case, the goal is to identify subsets of genes that characterize the different classes and to predict the outcomes for future samples based on their expression profiles. In the latter, the groups from which the observations arose are not known and the goal is to uncover the cluster structure of the observations and identify the discriminating variables. However, methods for analyzing longitudinal gene expression data are still relatively limited (Guo et al., 2003; Tai & Speed, 2006). We propose that the NCS develop new methods in the framework of functional data analysis and empirical Bayes analysis and treat the gene expression profiles over time as curves or functional data. Preliminary analysis of methods based on functional data analysis indicate such methods result in more sensitive procedures for identifying genes that show different expression patterns over time (Hong & Li, 2006; Leng & Mueller, 2006). We propose that the NCS generalize many commonly used multivariate analysis methods such as canonical correlation and correspondence analysis to the functional data for exploratory analysis and for graphically displaying the data. We also propose that the NCS develop regression analysis methods with functional data as predictors to account for gene expression dynamics over time. Functional data analysis provides a natural framework for accounting for gene expression levels measured over time and can potentially consider the dynamic nature of gene expression over time. These methods will be developed and made available to interested researchers on the NCS website.

10.5.6 Family Data

For population-based genetic association studies of complex traits, one of the potential areas of confounding involves the latent population substructures in the study population, which can result in the observation of spurious associations if such substructures exist and are not appropriately accounted for. Family-based study designs such as parent-child trios provide an alternative design for genetic association analysis of complex traits (Spielman & Ewen, 1996). For population-based case-control or cohort designs, genomic controls by typing a set of ancestry informative markers can be employed for adjusting for such population substructures in regression analysis (see more on this above).

Although deviations from the Hardy-Weinberg equilibrium (e.g., existence of migration) can identify systematic errors in genotyping, it is important to carry out other checks. For case-control studies of genetic association, under particular models for genotyping error there is no increase in type I errors of tests for genotype-disease associations (Gordon et al., 2002). (See Section 10.2.3 for a discussion of type I and II errors.) However, if general tests which ignore genotyping error are invalid, one solution is to integrate a realistic error model into association analysis for SNPs. A number of models for measurement error have been proposed and are described by Gordon et al. (2002) along with descriptions of how to appropriately test for association. Similarly, measurement errors in environmental exposures will be accounted for in the context of regression analysis with measurement errors (Ruppert et al., 2003, Chapter 15). Details of dealing with measurement error are provided in Section 10.3.

Genomic imprinting can be loosely defined as the gamete-of-origin dependent modification of phenotype. In other words, the phenotype elicited from a locus is differentially modified by the sex of the parent contributing that particular allele. This process results in a reversible gamete-of-origin specific marking of the genome that ultimately produces a functional difference between the genetic information contributed by each parent. In humans, the term genomic imprinting is usually described as mono-allelic gene expression or the inactivation of either the maternal or paternal allele of a particular locus. One important issue in the context of the NCS is to differentiate between fetal and maternal genotypic effects, which can be tested using the transmission test for linkage disequilibrium (Mitchell, 1997). While both parent-child triads and grandparent-grandchild designs can be used for testing maternal or parent of origin effect, the grandparent-grandchild design may in some situations provide higher power than the parent-child design (Weinberg et al., 1998; Wilcox et al., 1998). However, given the size of the NCS cohort, power should not be a problem (see Section 10.2.3, Power for Subgroups). In the framework of the log linear models as developed in Weinberg et al. (1998), one can also incorporate the environmental covariates into analysis of parents of origin and maternal-mediated genetic effects.

The parents-affected child trio design provides an alternative family-based design for studying associations between candidate genes and the risk of developing diseases or for studying gene-by-environment interactions. Designs based on the genotyping of affected individuals and their parents allow the detection of markers in linkage disequilibrium with disease genes (Spielman & Ewen, 1996). The main advantage of such a design as compared to population non-family cohort design is that it is free from the issue of spurious association caused by potential underlying population substructures. Such designs can be used for confirming the associations found in the standard population-based designs (non-family). Environmental covariates and gene-exposure interactions can be easily taken into account in the analysis (Li & Fan, 2000; Shih & Whittemore, 2002).

10.5.7 Multifactor Dimensionality Reduction and Issues of Multiple Comparisons

One important consideration with respect to genome-wide association studies is the issue of multiple comparisons. This issue usually arises in the context of hypothesis testing; however, even in exploratory studies without a formal hypothesis, there is generally an implicit hypothesis that a given discovered effect is zero.

When conducting multiple hypothesis tests, the type I error rate of 0.05 will hold for each individual test, but the overall probability of making at least one type I error is greatly increased. The most common procedure for protecting against this is to require a stringent "family-wise" error rate adjusted for the number of tests being conducted.

Although the family-wise error rate procedure is popular and performs well in genome-wide linkage analysis, it is too stringent for evaluating multiple loci, which may result in very low power. The false discovery rate (FDR) introduced by Benjamini and Hochberg (1995) provides a new notion of global error for multiple testing procedures. The idea of FDR is to use the expected proportion of false rejections of the null hypothesis among the total number of rejections as the measure of global error. Such a procedure leads to a global cutoff value that is adaptive to the data set (Sabatti et al., 2003). The FDR procedure will identify a lower cutoff level than the universal Bonferroni cutoff if a higher percentage of the null hypotheses tested are truly false. Such a procedure is most effective for the identification of loci with secondary effects. On the other hand, if all the null hypotheses are true (none of the analyzed markers is associated with the disease), controlling FDR is equivalent to controlling family-wise error rates. Although the original procedure by Benjamini and Hochberg was developed for independent tests and *p*-values, recent studies and extensions have indicated the procedure also works well when the tests are not independent, as might be expected in genome-wide association tests (Sabatti et al., 2003; Fernando et al., 2004).

Because health effects are commonly measured by multiple outcomes, the main tools we propose to apply or develop for the data to be collected are the regression models for multiple outcomes (Sammel et al., 1997; Sammel et al., 1999; Geys et al., 1999). We also propose to apply state-of-the-art models such as errors-in-variable models, missing-data methods, smoothing and methods for correlated data, such as longitudinal and spatial data analysis, to assess the health effects of dose, concentration, and duration of exposure. Semiparametric regression and generalized estimation equation methods can be developed for modeling the data and estimating the parameters in order to make fewer assumptions on the underlying models.

Another important application of multifactor dimensionality reduction is the investigation of gene-by-gene interactions. In logistic regression models, the number of possible interaction terms grows exponentially as each additional main effect is added (Ritchie et al., 2003). To reduce the dimensionality in data where interactions are the primary focus, one divides the data into training and test sets and then forms all possible permutations of the interaction terms. So for two genes, each with a dominant and recessive homozygote and a heterozygote, there are nine possible combinations. These combinations or interactions are treated as the "main effects" (Ritchie et al., 2003). Each cell class is labeled as high or low risk according to a predefined threshold. The MDR model that has the fewest misclassified individuals is selected and the process is repeated using 10-fold cross-validation to evaluate predictive ability and reduce spurious significances.

10.5.8 Twin Studies

The NCS cohort is estimated to eventually contain 3,000 twins. These twins will provide a unique opportunity to examine gene-by-environment interactions, especially with respect to complex diseases. Whereas monozygotic twins have identical genotypes, dizygotic twins have the same genetic variation as non-twin siblings. When monozygotic twins have one affected and one nonaffected twin, the ability to investigate gene-by-environment interactions with respect to specific SNPs, haplotypes and environmental factors will greatly facilitate the understanding of causal pathways.

Chapter 11

Data Use and Confidentiality Protections

11. DATA USE AND CONFIDENTIALITY PROTECTIONS

The National Children's Study Publications Subcommittee of the NCS Steering Committee will oversee the orderly and timely presentation of pertinent findings and data from NCS to the scientific and medical communities as well as to the public. This will include scientific papers, abstracts, and presentations. The subcommittee will also assure fair and equitable participation in the analysis of the data set and in the presentation of the study results by all NCS investigators.

Press releases and media interviews; and presentations to lay and community groups are the responsibility of the Program Office and Study Centers.

11.1 Disclosure Controls

The NCS Publications Subcommittee and the NCS Steering Committee will both have central roles in ensuring that study participants' data are appropriately protected. Methods to be used include a broad suite of disclosure control tools, which balance minimizing risk to participants with the potential for societal benefit. The ultimate goal is to protect individuals while still making data accessible to those who might make valuable contributions based on those data. There are several ways in which the NCS will strive to ensure such protection. First, the NCS will employ secure treatment of identifying data through limiting the appearance of personal identifiers on distributed data sets. Second, the NCS will control access to sensitive information by identifying different levels of access to the data and customizing data access plans across levels to ensure adequate protections on all releases of data. Third, the NCS will utilize statistical disclosure control procedures to reduce the appearance of unique personal information in the data that could result in re-identification of a participant.

11.2 Public Use Data Sets

Public use data sets for a given outcome and life stage will be developed for data sharing and made accessible to both the scientific/research community and the general public as soon as feasible, but no longer than within two years of the availability of a usable data set, and in accordance with NIH data sharing policy.[1] These types of public use data sets can be thought of as two levels of data.

11.2.1 Data Sets for the Scientific/Research Community

Data sets for professional researchers including academics, government workers, and others will be made available in compliance with the National Institutes of Health (NIH) data sharing policy:

> "Data-use sharing agreements will put some limitations on who can use the data and how they are to be used. Such agreements will contain requirements, including those to protect the privacy of subjects and the confidentiality of the data. These agreements will incorporate confidentiality standards to ensure data security at the recipient site and prohibit manipulation of data for the purposes

[1] Relevant NIH policy and guidance on data sharing can be found at the following websites: NIH Data Sharing Policy http://grants2.nih.gov/grants/policy/data_sharing/; NIH Data Sharing Policy and Implementation Guidance (Updated: March 5, 2003) http://grants2.nih.gov/grants/policy/data_sharing/data_sharing_guidance.htm

of identifying subjects. They will stipulate that the recipient not transfer the data to other users, that the data are only to be used for research purposes, that the proposed research using the data will be reviewed by an IRB [institutional review board], and the like."

Data made available to the scientific and research community will be available in de-identified data sets. Data will be released only after a full and detailed analysis of risk of data disclosure is performed. All users will sign appropriate confidentiality agreements.

11.2.2 Public Use Data Sets

Because there are no signed agreements or restrictions with regard to individual public data users, public use data files demand a very thorough initial review of the data for risk of disclosure. It is likely that a number of disclosure control techniques, jointly called perturbation, will be used on public data to ensure participants are fully protected from snoopers or inadvertent disclosures by those who are outside the research community.

11.3 Publication Policy

Study-wide publications from data that are not yet released to the general public or to the broad scientific/research community will emanate from a de-identified, validated data set issued by the Coordinating Center to NCS investigators. The data set will be available for analysis by NCS investigators after the completion of a life stage (e.g., completion of the 1-year visit) using data from either the entire cohort or from a random replicate of the entire cohort (wave of data collection). The NCS Community of Investigators consists of investigators in the Program Office, the Interagency Coordinating Committee, the Steering Committee, the Coordinating Center, and all Study Center principal investigators (and their site investigators).

A series of derived variables based on raw data, validated by the NCS Coordinating Center and approved by the Steering Committee (e.g., standardized or normed growth measurements), will be included in the database. Both core publications and non-core publications are anticipated.

11.3.1 Core Publications

Core publications are study-wide publications that address study methodology, baseline cohort descriptions, and the priority exposures and outcomes of the NCS (as identified in the 26 core hypotheses and updated over time). The scope of the core publications will be specified by the Steering Committee in collaboration with the Program Office. The NCS Publications Subcommittee will announce pending availability of data for each core hypothesis, and all interested members of the NCS Community of Investigators will be invited to submit proposals for analyses. Once formed, writing groups will be assigned a Coordinating Center statistician and may begin analysis after receiving approval from the Program Office to expend funds on the effort.

11.3.2 Non-core Publications

Non-core publications are study-wide publications not directly related to the Study's core hypotheses. Proposals for these publications will be generated by the NCS Community of Investigators as well as other government scientists (from lead agencies or otherwise). The Steering Committee and NCS Program Office may wish to advise or participate in the publication of non-core publications to ensure the maximal use of NCS data. Non-core analysis may be with or without collaboration with the Coordinating Center, resources allowing. The data access and publication proposal review process will be described in detail in the NCS Publications Subcommittee Policy Manual.

11.3.3 Approval Process

All members of the NCS Community of Investigators may request permission to publish from the Publication Subcommittee. If the proposal is rejected, the decision may be appealed. The approval process will be outlined in detail in the NCS Publications Policy Manual.

Chapter 12

Human Subjects Protections

12. HUMAN SUBJECTS PROTECTIONS

The National Children's Study is primarily observational in nature and will have both a low level of subject risk and a reasonable subject burden. However, the longitudinal nature of the research, the size and scope of the Study, and the diversity of the participants, make the human subjects protection issues significant. The NCS' commitment to collecting biologic, environmental, social, and behavioral measures and creating enduring data as well as biologic and environmental sample repositories with the potential for future studies not yet conceived, make the human subjects protections somewhat complex.

12.1 Study Population

The NCS will employ a national probability sample (see Chapter 6) with no exclusions based on gender, race, or ethnicity. Women, children, and men of all of the racial and ethnic groups and economic strata represented in the United States will be subjects. The rationale for this approach is to accrue and follow a population of children that captures the range and diversity of exposures and outcomes experienced by children in the United States.

Because a primary focus of the Study includes assessing the impact of exposures that occur early in pregnancy, three groups will be enrolled and followed: pregnant women of any age and their husbands/partners; adult women planning pregnancy; and adult women not planning pregnancy but with some likelihood of becoming pregnant. All births to mothers who meet the eligibility criteria will be included.

Women who are cognitively impaired or mentally ill are not eligible if they are not able to understand fully the Study's requirements and to grant informed consent. Only women with the capacity to consent will be enrolled.

At the time when a pregnant woman is enrolled in the Study, the biological father will also be invited to participate. If an enrolled woman does not want to identify or does not want the Study to contact the biological father, the Study will not contact the father. In these instances, the pregnant woman and her child would still be eligible for participation. The father does not need to live in the same home as the mother for initial inclusion in the Study, however, there are no plans to follow biological fathers or biological mothers who have no contact with the child.

Families that move will be followed to minimize the number of participants who are lost to follow-up. Because all births to mothers who meet the eligibility criteria are eligible for the Study, there will be children in the Study born to surrogate mothers, children who will be adopted, children who will be assigned to foster homes, and children whose mothers are on active duty in the military. In addition to children whose families move, foster children, adopted children, military children, and children whose parents divorce, may change households after birth. Because the children are the primary participants, they will be followed if they move or otherwise change households. The Study will use information collected from participants, as well as publicly available data, to track and locate families and children in the Study who change households.

12.1.1 Strategies/Procedures for Recruitment

Strategies for recruitment are outlined in detail in Chapter 6. The primary approach involves screening and recruitment from households located in neighborhoods targeted for inclusion in the Study

and through providers of prenatal care. A variety of materials and strategies, including, but not limited to, media outreach and distribution of brochures and newsletters, will be utilized to increase public awareness of the Study and aid with recruitment of Study subjects.

12.1.2 Special Classes of Research Participants

12.1.2.1 Pregnant Women and Fetuses

The NCS will recruit and follow women prior to and during pregnancy. The NCS fulfills the requirements for research involving pregnant women and fetuses as described in section §45 CFR 46.204 of the Code of Federal Regulations, subpart B. The purpose of the NCS is to develop important biomedical and psychosocial knowledge about the impact of biologic, environmental, social, and behavioral exposures prior to and around the time of conception, during pregnancy, and as the child ages, on the future health and development of children. This information cannot be obtained by other means. Risks to the women and fetuses are not greater than minimal, and the research will in no way affect medical decisions about pregnancy management and outcome. Provisions in this section of the regulations also state that consent from the father of the fetus is not necessary when the research imposes only minimal risks to the fetus.

12.1.2.2 Pregnant Adolescents

The NCS will enroll pregnant adolescents who are identified during the household screening or through sites of prenatal care, and who are otherwise eligible for participation in the Study (e.g., first trimester of pregnancy). Women younger than age 18 will not be eligible for inclusion in the preconception cohort. Laws regarding the legal status of pregnant adolescents vary by state. In some jurisdictions, pregnant adolescents are considered "emancipated" from their families and can be treated as adults for the purposes of obtaining informed consent for this research project. Additionally, in many jurisdictions pregnant adolescents may legally seek medical care for pregnancy without involving their parents. In these jurisdictions, Institutional Review Boards (IRBs) may permit pregnant teens to consent to participation in research in studies such as the NCS without parental involvement. Finally, even in jurisdictions where pregnant adolescents are not considered emancipated or able to consent for their medical treatment, IRBs may waive involvement of parents in the informed consent process under certain conditions [Section §45 CFR 46.408(c)]. Local centers, in consultation with their IRBs, will determine whether parental permission is required in addition to the consent of the pregnant adolescent under the age of majority.

12.1.2.3 Children and Adolescents

Investigating the effects of environmental exposures and gene-environment interactions on the outcome of pregnancy and on the growth and development of children is the primary aim of the NCS. Thus, children from newborn to adulthood will be the subjects of this longitudinal Study. Each child's parent or guardian will be asked to grant permission for participation in the Study. It is the expectation that children, as young as toddlers and continuing through adolescence, will be informed about the Study and its goals in developmentally appropriate language, using creative methods such as newsletters, comic books, Web sites and DVDs. IRBs will receive all informational materials for review and approval prior to implementation. Issues related to consent and assent are described below.

12.1.2.4 Economically or Educationally Disadvantaged Individuals

It is anticipated that some of the participant families in the NCS will be economically or educationally disadvantaged. Section §45 CFR 46.111(b) of the federal regulations requires the IRB to assure additional safeguards are provided in a study when some or all of the subjects are likely to be vulnerable to coercion or undue influence because of economic or educational disadvantage. The NCS will design additional safeguards into the recruitment and retention activities for all participants to encourage informed participation of all eligible subjects. Each Study Center will be required to develop meaningful and enduring partnerships with the communities from which participants will be recruited. These activities along with the informed consent process described below will result in no coercion or undue influence on potential participants.

12.1.2.5 Foster Children and Wards of the State

Because of the subject matter of interest to the NCS and the probability-based sample design, it is important that every eligible child be enrolled and retained in the Study. Some children eligible for enrollment in the Study may be in foster care, may be wards of the state, or may transition into these arrangements at some time after enrollment in the Study. Permission for continued participation of the child in the Study will be sought from whatever administrative agency or institution is responsible for the care of the child and, in addition, from the foster parent. (See Section 12.6.6 for details on consent for participation of foster children and wards of the state).

12.2 Benefits

Although it is possible individuals may benefit from participation, the Study does not claim that participants will have the "prospect of direct benefit" from the Study. There are likely to be collateral benefits of participation, including information about individual examinations and tests performed during the course of the Study, health education, increased awareness of medical and social services available in the communities studied, and serendipitous findings of clinical relevance or of predictive value to participants and their families.

The potential for NCS to benefit society and children in general is extraordinary. The hypotheses being addressed and the data being amassed for future analyses are likely to impact the health and development of children for decades to come.

12.3 Potential Risks

Each of the procedures, measurements, and assessments in NCS is designed to fulfill the definition of "minimal risk" in the federal regulations [§45 CFR 46.102(i)] and to be reviewed by IRBs under §45 CFR 46.404 "Research not involving greater than minimal risk." Minimal risk as defined in the federal regulations means "that the probability and magnitude of harm or discomfort anticipated in the research are not greater, in and of themselves, than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests." In addition, the NCS staff is committed to minimizing risks even when the risks are minimal. Well-trained and competent individuals who have experience with pregnant women and children of the appropriate age will perform each procedure that might include discomfort or pain, such as a blood stick. Settings in which tests will be performed or information will be obtained from women and children will be woman- or child-friendly and respectful of the participants' needs and privacy. Questionnaires will be structured to avoid creating

discomfort for the women or children; and participants will be reminded at each data collection encounter that their participation is voluntary, they have the right to withdraw from the Study at any time, and they may refuse to answer or may skip any question.

There are additional issues associated with the Study related to testing and storage of biologic specimens and environmental samples; reporting concerns regarding possible child abuse or neglect; possible breaches of confidentiality; and informing participants of Study findings (which potentially could result in psychological effects, such as anxiety, or could have a financial impact, such as costs for additional testing). Each of these has been considered by the Study, and plans are in place to protect the welfare of participants and families involved in the Study.

The NCS staff is cognizant that while research staff is in and around the homes of participants, they may observe or learn about environmental hazards or behaviors that place a child in imminent danger, and investigators may be legally required to report such observations or information to specific authorities in some jurisdictions. The NCS staff feels morally obligated to respond to protect the interests of children when they are found to be in serious imminent danger, even if there are no reporting laws. Thus, the informed consent process will inform participants that if a data collector observes a child is in imminent danger of serious harm or the subject of child abuse, the information will be reported to the proper authorities to obtain help for the child. Study procedure manuals and interviewer training will describe the process that will be invoked to report such observations to the principal investigator or his/her designee at each site. Primary data gatherers will be trained to note such dangers to participants and inform their supervisors immediately for evaluation as to the proper course of action. It will not be the sole responsibility of the data gatherers to report the observations to authorities; rather it will be the responsibility of the professional staff under the supervision of the principal investigator to assure reporting is performed in an appropriate and timely manner. Study Centers will each have knowledge of local resources including social service providers for referral purposes. Each Study Center will develop a local mechanism for this reporting and referral process.

Primary data gatherers will also be trained to respond to observations of adults in danger, such as domestic violence between adults or suicidal tendencies. The NCS staff has a moral responsibility to assist adults in dangerous situations, but these situations will be dealt with differently. The adult victims will be involved in the process, and no reports will be filed with any authorities without the involvement and approval of the adult victims, unless required by law. Names of social service referral agencies will be provided upon request to adult victims of domestic violence.

12.4 Adjunct Studies

It is anticipated that in addition to the core protocol for the NCS, there will be adjunct studies proposed and conducted by investigators associated with the NCS. Such studies will involve a subset of the NCS cohort, at one or more Study Centers, on all or a portion of the local participants or their data. To protect the quality and integrity of the NCS, adjunct studies will be reviewed and approved through a defined process involving formal review and approval (see Chapter 16 for details).

Since Study participants may be asked to participate in these adjunct studies, the Study consent process will include a statement that participants may be contacted for other studies connected with NCS as a result of their participation in NCS, but they are not obligated to participate in any of these adjunct studies. All adjunct studies that involve additional interaction with human subjects will require IRB review and additional informed consent.

12.5 Incentives and Compensation

Recruitment and retention for the NCS will be a significant challenge in light of the respondent burden and the long-term commitment required of participants. It is expected that reasonable incentives will be part of the strategy for recruitment and retention of participants.

Compensation for participation will include reimbursement for expenses incurred in research participation such as travel to and from the research centers, parking, etc., and reasonable payment for time spent in participation in the research (approximately $25 to $50 per visit or exam, depending on the amount of time and effort involved). Adult participants and older children will be compensated for time spent completing questionnaires, for providing biologic specimens, and for other Study activities.

Small "gifts of appreciation" for continued participation will be provided to participants periodically. These may include token items such as T-shirts, tote bags, toiletries, books, and CDs. Gifts will not have sufficient monetary value as to unduly affect the voluntariness of consent to participate or of continued participation in the Study.

12.6 Consent and Assent Processes

The informed consent process will begin when potential participants are first notified about the Study and will vary depending upon the ages and types of participants and the pregnancy status of women. The first step in the process will involve advance mailings of Study material to potential participants. These materials will include a letter describing the Study. The next step will be enumerating household members. Then, pregnancy screening will be performed with all eligible females. Pregnancy screening will involve a script and a computer-assisted self-administered interview and will only include what is needed to determine Study eligibility. The Study will ask IRBs to accept oral consent for this process, because the eligibility screening will only involve questions about criteria used to determine the eligibility status of potential participants and about age in order to determine which consent process to administer. If a participant is found to be eligible and is willing to participate, only then will the full informed consent process commence.

The informed consent plan for the Study takes into account the types of participants and is tailored to address specific issues pertaining to each type. Women will provide informed consent during pregnancy for themselves and their child. There will not be a new consent process specifically for the baby at the time of birth.

The types of participants providing informed consent, or in the case of young children, assent will include:

- Adult women at risk of becoming pregnant (preconception women)
- Pregnant women (adult and adolescent)
- Biological fathers
- Other caregivers
- Children (through the phases: young children, adolescents, young adults)

The consent plan recognizes there will be transitions for some participants between types, and these transitions will affect the consent process. For example, preconception women might become pregnant and will need to provide additional consent for their own full participation in the Study and for the participation of their children. The assent/consent process for children will also change as the children grow from young children, to adolescents, to the age of majority.

The informed consent materials anticipate low literacy, they are culturally sensitive, and they reflect the diversity of potential participants. As part of the informed consent process, there is a method, described below, to ascertain if the participant understands key elements of the Study and what is involved in participation.

All consent materials will be available in English and Spanish, and other translations will be available as needed. Interpreters will be available for additional languages. A copy of the informed consent document will be made available to the participants electronically and as a paper copy.

12.6.1 Electronic Audio/Video Consent Tool Pilot

A video approach to informed consent is being developed to address some of the challenges with the traditional method for obtaining informed consent, as well as to provide a means for assuring consistency in the informed consent procedures across multiple sites of implementation. The primary goal of the tools is to enhance prospective participants' understanding of the purpose of the Study and all of the essential elements of informed consent. The videos take into account the diversity of potential participants and the reality that some eligible participants may have low literacy. They also accommodate the hearing impaired through closed captioning. There are separate versions of the tool for each of the different types of participants (preconception [nonpregnant] women, pregnant women, and biological fathers). During the Study's pilot phase, a computer-based interactive video informed consent tool will be compared to traditional written informed consent. The two methods of obtaining informed consent will be compared both in terms of understanding of the Study requirements (content of the consent) and Study enrollment.

This audio-visual presentation will be shown on the data collector's laptop or tablet computer. Study staff will be present during the entire informed consent process to assist with the computer presentation and answer participants' questions. The presentation includes embedded questions that assess the participant's understanding of what they have seen and heard to help ensure they understand the key elements of the Study and what their participation will involve. If the participant does not answer a question correctly, the presentation provides additional information and chances until the participant selects the correct answer. In that way, participants will not be excluded if they fail to answer some of the questions correctly. To consent, they will be required to keep trying until they understand which answer is correct, and the presentation will explain why the answer is correct to reiterate the information. The participant's written signature will be obtained electronically at the end of the presentation. A written copy of the material described in the informed consent video will be left with each participant.

12.6.2 Women Age 18 and Older

The NCS will initially recruit women ages 18 and older prior to and during pregnancy. Potential participants will be told that they can share the consent materials and discuss participation with family, friends, and, if they choose, their physician before deciding whether to enroll. Local research staff will be available in person to answer any questions and clarify any aspects of the NCS.

12.6.3 Women Less Than 18 Years of Age

Women younger than 18 who are pregnant will be eligible for the pregnancy portions of the Study. Special procedures will be used for women younger than 18 to ensure that encouragement to participate will not be undue or interpreted as pressure. There will be age-related differences in monitoring women for pregnancy. The Study will not enroll those younger than 18 who are not pregnant, and these young women will not be asked whether they are planning to get pregnant.

The consent process for pregnant women under the age of majority will be consistent with the laws of the local jurisdiction. Generally, federal regulations permit pregnant women of any age to consent for minimal risk research for themselves and their children. If the pregnant young woman is between 15 and 18, she will be encouraged to consult with her family prior to providing informed consent for herself and her child. If the pregnant young woman is younger than 15, then the Study protocol will require the consent of her parent or legal guardian. The young women will be asked to enroll and to provide informed consent for themselves and for their children.

12.6.4 Assent of Children

Consistent with §45 CFR 46.408(a), it is the intention of the NCS to obtain assent for participation in the Study from children beginning at approximately age 7 if they are developmentally and cognitively able. Each child who is developmentally and cognitively able to assent to continued participation in the Study will be approached. The process for obtaining and documenting "child assent" will be presented to each participating IRB at least one year before the first subjects of the Study will become 7 years old. The description of this process will include the methods that will be used to determine if a child is developmentally and cognitively competent to be approached for assent.

All children enrolled in the Study will receive continual updates on the progress of the Study through developmentally appropriate newsletters, Web sites, and other communications. They will be encouraged to continue to participate in the Study, answer questionnaires, and attend scheduled follow-up visits.

An "adolescent assent" process will be developed to obtain the affirmative agreement of each teen to continue participation in the Study. This process will be initiated at approximately age 14. A description of this process and the methods to obtain and document assent from developmentally and cognitively capable teens will be provided to each participating IRB at least one year before any of the subjects turn 14. The description of this process will include the methods that will be used to determine if an individual adolescent is developmentally and cognitively competent to be approached for assent.

12.6.5 Consent of Adolescents (When Child Participants Reach Age of Majority)

As adolescent participants in the NCS reach the legal age of majority in each jurisdiction (generally age 18), a fully informed consent will be obtained from each participant for continued participation in the Study and for continued use of stored samples for analysis. A consent process for these adult subjects will be developed and submitted to the IRB at least one year before participants turn 18.

12.6.6 Foster Children

Foster children who are wards of the state are permitted to participate in research without any additional procedural safeguards when study risks are minimal (§45 CFR 46.409). Only when the research involves greater than minimal risk and no prospect of direct benefit is there a requirement for the IRB to provide additional procedural safeguards through the appointment of an advocate. Because the NCS is primarily an observational study with a minimal level of risk, no such procedural safeguards should be required. Because knowledge about the child's living environment is essential to the Study, in addition to obtaining consent from the agency responsible for the child, a foster parent will be approached to give permission for their participation and the child's continued participation in the Study. The foster parent will be fully informed about the purposes and procedures involved in the Study, and informed consent will be obtained for their participation (as caregiver), and for continued participation of the child.

12.7 Revealing Findings to Participants, Families, and Communities

Revealing some of the Study data findings to individual participants is seen as an ethical obligation but may also be an important recruitment and retention strategy. Revealing local aggregate findings to the communities is seen as an important strategy to maintain community engagement.

12.7.1 Revealing Individual Findings to Participants and Families

Some routine physical and laboratory test results will be revealed periodically as an incentive to participation. For example, results of routine physical measurements (e.g., height, weight, and blood pressure) and routine laboratory tests performed on biologic samples (e.g., hematocrit) will be provided to participants on a regular and recurring basis. These results will be presented in a context that allows the participant to compare their individual results with normative data when appropriate (e.g., growth curves, normal range of hematocrit for age).

Unless clinically relevant and actionable, NCS generally will not provide genetic information and other medical information to participants or family members. Much of the data collected in the NCS will be of uncertain relevance to the health or well-being of individual participants, and relevant for research purposes only. Participants will be informed of this during the consent process.

If clinically relevant and actionable medical information that may impact the health of the participants is found, they will be advised of that information. Participants may opt out of any measurement, test, biological specimen collection, or environmental sample collection. However, if a test, measurement, or collection is performed, and the results indicate a known health effect or risk to the participant that is clinically relevant and actionable, the Study is obligated to reveal the finding to the participant.

If clinically relevant and actionable genetic information is found in the future, participants will be informed that such information exists and may be obtained upon request. If participants request the information, NCS staff will explain to the participants the consequences of learning such information, and if the participants still desire the information, NCS staff will inform the participants in a sensitive and knowledgeable manner.

Results of environmental sample analysis will only be revealed to participants if there is a known and generally accepted risk relation between the exposure and a significant negative health outcome. This includes the following situations:

- There are state requirements to disclose (e.g., elevated blood lead or mercury concentration).
- Federal or state standards or guidelines exist.
- Appropriate risk assessment that has been conducted and published is applicable to the community in which the samples were collected (e.g., lead levels in dust or soil).

Environmental sample results provided to participants will be accompanied by an explanation and context for the result, basic information about the sources and risks of the chemical/agent, and guidance on where to find more information.

12.7.2 Revealing Aggregate Findings to Participants and Communities

The NCS is also committed to informing participants about aggregate data on a periodic basis as Study findings unfold. Because environmental findings may reveal local problems that could impact property values, etc., there may be potential risks to individuals, (participants and nonparticipants) and to the entire community, of revealing information found in the Study. Therefore, revealing information to communities must be done thoughtfully and with some level of preparation. The NCS will always inform individual participants living in a community of any personal findings of concern before informing communities of the findings.

To help keep participants engaged in the Study, all participants enrolled in the Study, adult and child, will receive periodic national updates on the progress of the Study through newsletters, Web sites, and other media. Web sites will be developed for the adults and for children and adolescents of various ages. This continual process will include updates on the progress of the Study, health information appropriate for all participants, some insights into how large studies such as NCS analyze findings to make inferences about how an exposure might be related to an outcome, and serially, information about the Study's findings.

Each site will also integrate a local process into this national process to reveal some of the aggregate findings to the local community, to maintain contact with participants, to give site-specific information to communities and participants, and to help maintain community engagement.

12.8 Biobanking and Environmental Sample Banking

Biologic specimens will be collected from women during the preconception period, during pregnancy, and after birth. Specimens will also be collected from biological fathers (during the pregnancy period) and from the child serially after birth. At the time of birth, collection of cord blood and placental material is planned. HIV testing is not currently planned for NCS.

The NCS plans to obtain biologic specimens from participants including blood, urine, saliva, breast milk, and small samples of hair. These specimens will be used to measure various physiologic parameters (e.g., hematocrit, iron stores) and environmental exposures (e.g., lead, chemicals), and to provide genetic information about each participant. Sample volumes will be kept minimal and all child

blood samples will be less than 5 milliliters per kilogram body weight. Specimens will be analyzed and/or stored in one or more repositories for future studies.

Periodically, the NCS will also collect environmental samples of air, dust, water, and soil from the homes of participants and other places where the child spends more than 30 hours per week. These samples will be analyzed to determine and measure environmental exposures and/or will be stored in one or more repositories for future studies.

Effects of environmental exposures on gene expression are among the most important interests of the NCS. Therefore, biologic specimens for DNA analysis will be obtained from participants. The NCS is cognizant that human genomic data are private, intimate, sensitive, and create special concerns about the potential for discrimination, stigmatization, and impact on future employment or insurance. The informed consent process will include reference to the reasons and importance of obtaining genetic information on each participant.

To protect the confidentiality of participants, only unique identification numbers without personal identifiers will be used for all biologic specimens collected and all information derived from those specimens. Data that can be used to link the specimens to personal identifiers and to other data obtained from individual subjects during this longitudinal study will be maintained separately, securely, and confidentially. To further protect participant confidentiality, the NCS will obtain a federal Certificate of Confidentiality through the National Institute of Child Health and Human Development from the U.S. Department of Health and Human Services. The Certificate of Confidentiality will protect the data from forced release through a court subpoena.

PART III
STUDY MANAGEMENT AND SUPPORT

Chapter 13

Information Management System (IMS)

PART III: STUDY MANAGEMENT AND SUPPORT

13. INFORMATION MANAGEMENT SYSTEM (IMS)

13.1 Introduction

The Information Management System (IMS) is integral to the National Children's Study. The IMS houses all NCS-related information and serves investigators and the public throughout the Study lifecycle. At the earliest stages of the Study (study design, recruitment, and enrollment of expectant mothers and the Study launch), the IMS records and tracks enrollment, personal information, and informed consent. Through pregnancy, birth, childhood, and adolescence, it supports the tracking of participants, collection of data, report of findings, and incentive management. As Study Centers and data collectors collect biological data and samples, physical measures, environmental data and samples, and questionnaire and assessment data, and as laboratories analyze those specimens, the IMS records, transforms, analyzes, reports on, and protects the information. The IMS also maintains information regarding the location and disposition of physical samples and the results of the sample analysis. To facilitate data gathering, the IMS assists in scheduling visits, including generation of visit reminders to participants and schedules of upcoming data activities for data collectors. Prior to going into the field, the data collectors upload all data needed to conduct interviews and assessments, including participant and schedule information.

Critical features of the IMS include its ability to collect, to store, and to report on the data during the Study as well as to store and report on the data after the Study is complete. To reduce the risks associated with data collection, storage, and reporting, data storage for the Study will be centralized in the IMS at the Coordinating Center. Data are gathered through multiple means (such as laptop-based survey instruments, Web-based interfaces, and measurement devices) and are electronically sent to or entered into the IMS. Backup and protection of all data are guaranteed by the centralized storage. The IMS supports centralized, uniform, high quality data collection and analysis activities for the Study. The IMS also supports uniform and consistent participant de-identification and strong controls over re-identification, as well as producing investigator-specific data sets.

Since critical Study activities are supported centrally, the IMS maintains continuous "24x7" Study operations. It incorporates state-of-the-art redundancy, fault tolerance, and disaster recovery mechanisms to ensure that operations can continue if hardware, software, or communications fail. The majority of IMS functionality is accessible to the Study Centers through an Internet browser over a secure network. Other functionality is accessible through disconnected data collection devices (e.g., laptop computers used by field data collectors).

Data collection in the home or at other field locations utilizes laptop computers and, occasionally, environmental sampling devices. This collected information is synchronized with the central database when the data collector is able to log into the Coordinating Center (either remotely or through a direct connection in a Study facility).

Clinical event data collection regarding such data as diagnoses, interventions, etc., which occur over time, constitute important outcomes and exposures to incorporate into the participant data base. Methodologies are being studied to facilitate obtaining these data during the Study from disparate sources such as primary care physicians, specialty consultants, hospitals, emergency rooms, and public health clinics.

13.2 Security and Privacy

Security and privacy are factored into every aspect of the IMS design. Security includes protection of sensitive data from corruption, theft, tampering, or unauthorized use, as well as protection from loss or corruption due to internal problems (e.g., a hardware or software failure) or external forces (e.g., a natural disaster). Privacy restricts access of sensitive information to only those individuals who are authorized to use or to view such data. De-identification of data—separating potentially identifying personal information from the actual participant data—is one aspect of privacy.

The IMS is hosted on a number of dedicated servers in a secure facility where physical access to the servers is restricted only to authorized personnel. The Study data are stored and managed in a secure network environment that is protected by continuously updated firewall, anti-virus, and anti-intrusion hardware and software. Systems are actively monitored to detect and block any attempt at intrusion or "hacking." Secure network connections are established between the Coordinating Center and external entities (e.g. Study Centers, labs, and repositories) to ensure data are not compromised during transmission. All data are encrypted during transmission and upon storage.

The IMS complies with various policies and regulations, such as the Health Insurance Portability and Accountability Act (HIPAA), to protect the privacy of the participants. Even Study staff, such as the Coordinating Center data managers and analysts, are not able to associate Study data with actual participant identities except under strictly defined conditions. To fulfill this mandate, the IMS employs a second layer of security specially designed to segregate participants' personal identifying data (PID) from the rest of the data. PID is stored in specially encrypted databases on servers that are physically separate from the main database servers. User IDs, passwords, and "digital certificates" allow access to PID only by authorized individuals and from authorized access points. The password/digital certificate system may also be augmented by a biometric identification technology such as thumbprint scanning to guarantee that any request for PID is genuine and coming from an authorized user.

13.3 Architecture/Framework

Since the Study will last more than 20 years, the IMS is designed with the ability to grow with the Study and to adapt with the evolution of technology. The IMS framework allows reusing existing applications and systems while accommodating future technology expansion. The framework accomplishes this by focusing on interoperability and component-based architecture.

Interoperability is defined as the ability of different types of computers, networks, operating systems, and applications to work together effectively to exchange information in a useful and meaningful manner. Interoperability requires not only the ability to transfer data, but a common understanding of what those data mean. The IMS supports interoperability with other systems (e.g., external databases with relevant data) by including multiple methods of transferring data between systems and by the use of industry standards to define not only the syntax but also the meaning of the data. Leveraging standards for integration enables the IMS to be flexible when future technology changes are implemented.

The IMS is a component-based architecture in which "components" (e.g. system building blocks) are responsible for specified functions. These components have well-defined interfaces. This approach supports later replacement of a component with newer or alternate versions that enhance functionality or incorporate new technology (in a "plug and play" manner). The result is a scalable IMS adaptable to the Study's long-term goals.

Chapter 14

Adverse Event Reporting and Data Monitoring

14. ADVERSE EVENT REPORTING AND DATA MONITORING

14.1 Monitoring Subjects and Criteria for Withdrawal from the Study

The National Children's Study is relatively noninvasive, and the research protocol has no interventions. The Study and all procedures are also of no more than minimal risk. Thus, there are no conditions envisioned, either due to Study procedures or unrelated to Study procedures, which would preclude continuation in the Study. The only situation in which we would discontinue follow-up with the family is if there is a pregnancy loss or an enrolled child dies, and the occurrence is beyond the enrollment period (such that it is beyond the point when subsequent pregnancies would be eligible for enrollment in the Study). If Study subjects develop a condition that renders them incapable of providing the continuing informed consent required of the Study, continuing consent will be sought from the legally appropriate party.

Any participant may withdraw from participation in the NCS at any time. Declining participation or withdrawing from the Study will in no way affect their relationship with the local research sites or associated medical institutions. In the event a participant withdraws from the Study or the Study is unable to locate a participant (lost to follow-up), data and samples obtained to that point will be maintained for use in future analyses unless the participant requests the samples be discarded and not used in any future analyses. Participants will also be allowed to stop participation in the Study for brief periods and then rejoin in the future. If a participant dies, all data will be maintained in the data sets for all subsequent analyses. Participants will be informed of these policies.

14.2 Data and Safety Monitoring Board

A Data and Safety Monitoring Board (DSMB) consisting of 5 to 10 individuals not associated with the NCS will be created to review data periodically. The DSMB will have expertise in biostatistics, epidemiology, environmental toxicology, pediatrics, genetics, psychology, social determinants of health, ethics, and other appropriate disciplines. The DSMB will report to the Study Director and review standard process data such as accrual rates and adverse events and possibly other appropriate aspects of study data as determined by the Study Director and the Steering Committee. The DSMB will alert the Steering Committee if data become available that might require participants to be informed about the finding. An Ethics Advisory Committee (Subcommittee of the Federal Advisory Committee to the Study) will be established to review relevant situations at the request of the NCS Study Director or the NCS Steering Committee.

14.3 Ethics Advisory Committee

During the course of the NCS, environmental findings may reveal information that could be relevant not only to participants but also to members of the community from which participants have been recruited. The Ethics Advisory Committee of the NCS will assist in considering which information is of this type and will be available to assist the regional sites, in partnership with their local community advisors, to develop a strategy for dissemination of this information in an appropriate manner.

Chapter 15

Quality Assurance and Quality Control (QA/QC)

15. QUALITY ASSURANCE AND QUALITY CONTROL (QA/QC)

15.1 General Approach

Because the National Children's Study is a multi-site, multi-year study involving the collection of complex data as well as physical/medical measures, environmental samples, and biological specimens, quality assurance and quality control (QA/QC) are vital to ensure the data, measures, samples, and specimens are collected correctly and consistently across all sites and throughout all years. All NCS partners have long-standing reputations for conducting high-quality scientific research. However, as with all large multi-center studies, standard QA/QC mechanisms and procedures must be developed and utilized to assure data quality, integrity, completeness, and comparability throughout the Study. To accomplish this, a Quality Management Plan (QMP) and a Coordinating Center QA/QC plan will be developed, applied, maintained, and updated as needed throughout the Study. The QA/QC plan will specify QA/QC procedures and policies that will apply to Coordinating Center operations and also QA/QC procedures and policies that the Study will require of the four types of collaborators: Study Centers, laboratories, clinical testing facilities, and central repositories.

An individual will be assigned by the NCS Program Office to serve as the NCS Program Office Quality Manager. This individual and, as the study grows, his or her staff, will provide independent high-level oversight of QA/QC activities conducted by the Coordinating Center, Study Centers, Information Management System (IMS) contractor, laboratories, clinical testing facilities, and repositories. Examples of the types of activities the Quality Manager will perform include conducting independent audits/assessments of the NCS components, reviewing audit reports, and making recommendations to the Study Director and Project Officers on corrective actions.

The major tool for Study and data management, and thus Study QA/QC, will be the IMS. The IMS will not only provide an array of QA/QC monitoring functions, it will also track the QA/QC activities. There are three aspects of QA/QC related to the IMS: (1) the IMS will capture all Study data allowing for Study oversight of data completeness and production; (2) the IMS will capture all Study QA/QC data allowing for Study oversight of data quality, integrity, and comparability; and (3) specific QA/QC checks will oversee the IMS.

15.2 QA/QC Activities for IMS

The IMS is the computerized heart of the Study. It must support the collection, management, and storage of the study data and manage highly complex study activities involving thousands of staff and participants across the country. The electronic edits built into the IMS will be the key to ensuring data quality, and the monitoring and tracking systems it includes will be critical to ensure proper Study management. Since the NCS will be underway for such a long period of time, it is critical that the IMS be able to accept upgrades and new technologies without going out of service. These challenges require constant attention to quality.

15.2.1 IMS System Quality

The IMS will not be able to ensure the quality of NCS data unless it is itself a quality system that performs reliably, accurately, and according to specification. System quality begins with the team that builds and integrates it. A fundamental component of the quality system for the IMS is a contractual requirement that the IMS contractor must maintain ISO 9001:2000 certification and demonstrate CMM

Level 3 compliance on an ongoing basis. During the planning phase, the IMS contractor developed a CMM plan which defined internal roles and responsibilities, record-keeping requirements, and other quality-affecting parameters. The CMM plan ensures the organization's software development processes include visibility, oversight, and checkpoints throughout the software development life cycle.

It is an axiom in software development that the earlier an error or problem is found, the easier and cheaper it is to correct. Thus, the IMS contractor will perform early reviews of requirements and design specifications, working closely with Program Office and Coordinating Center staff, who are the subject matter experts. This process ensures that when software is built (or purchased commercially) and integrated, it will meet requirements. Source code reviews during actual development will further ensure that errors are caught as early as possible.

A key step in ensuring system quality is testing. The IMS will be tested in four stages. In the first stage, the developers and integrators will test each hardware and software component to ensure it functions according to specifications, a process called "unit testing." In the second stage, and in a separate process, an independent test team will examine the IMS requirements documents and prepare a detailed test plan for each IMS system. After initial training by the development team, the independent test team will execute the test plan against each of the systems, identifying problems and placing them into a formal defect tracking system. Each problem will be prioritized and tracked to resolution, and the systems will be retested until they pass. The third stage of testing will be performed by Coordinating Center and Study Center staff who will test each of the IMS systems using real-world study scenarios to determine if the systems perform their functions properly. This process is known as "acceptance testing," and systems cannot be fielded until they pass. The final stage of testing is ongoing. Whenever a system is enhanced, upgraded, or a defect is found and corrected, not only must the new or changed elements be tested, but also a "regression test" must be performed by the independent test team to ensure the changes do not adversely affect other functionality.

Throughout the Study, the Coordinating Center will capture, track, and report IMS infrastructure outages as well as software defect reports, IMS help desk calls, and application error logs to compute an ongoing reliability factor that will be reported monthly and yearly. The IMS will include application event logs that will capture application failures along with reporting capability. In the unlikely event of a system outage, the Coordinating Center will document the outage with an incident report that describes the cause of the outage, the measures taken to resolve it, and the processes and procedures that can be implemented to prevent a similar future occurrence. Prior to implementation, the Coordinating Center will confirm the computation of the reliability statistics with the NCS Program Office.

15.2.2 IMS Data Quality

The IMS will be designed to maintain and ensure the quality of the NCS data throughout its life cycle from collection through analysis, storage and eventual archive. Quality, in this context, is defined in four broad dimensions. A data element must be:

- collected accurately;
- protected from tampering or inadvertent alteration or corruption;
- traceable and attributable to its original source; and
- associated with audit trails and decision logs that document all changes to it as well as the source and reasoning behind each change.

To support accurate data collection, the IMS will maintain calibration and test records for data collection devices and instruments, including questionnaire instruments. The IMS will also maintain records documenting data collectors' training and certification. To support data security, the IMS security features will include many technical and procedural checks and guards to protect data from tampering or corruption, including encryption, network firewalls, multi-factor authentication of users, and role-based access controls. To ensure that data are always traceable and attributable to source, each data element will carry associated metadata to document its history and context. IMS data management systems will use audit trails, include timestamps, and will identify the source as well as the nature of data changes. Decision logs will document the reasons behind any changes made to data post-collection as well as larger study-level decisions that may cause wholesale instrument or methodology changes.

The IMS will not only maintain data quality, it will provide the information needed to make improvements in data collection instruments, methods, and techniques. Edit reports produced by the IMS will document edit failures and their resolution. The IMS will use the edit failures to identify possible data collection or manipulation or metadata errors that will be used to compute an overall accuracy statistic for data collected by the IMS.

In addition to system reliability and accuracy statistics, reports and audits will be used to assess the quality of the IMS products at any given time in the project life cycle. Reporting on defects and change requests provides some useful quality indicators, such as team productivity and bottlenecks, evaluation of workload distribution, the need to insert more or less flexibility into processes, and overall schedule progress. Audits provide verification that processes are being followed and that traceability exists between coded software and requirements or change requests.

15.3 Training Data Collectors

Comprehensive training of Study Center data collection staff will be an important aspect of the QA/QC plan. Highly experienced Coordinating Center staff will develop and implement a carefully designed and thorough training program, including training manuals, training exercises, role-play scenarios, audio/visual tools, and certification procedures. The Coordinating Center staff will conduct initial training of Study Center staff using a "train-the-trainer" method to prepare the Study Center staff who will subsequently conduct the training and retraining for data collection at their site. The training sessions and materials will be structured around specific competency-based objectives using a variety of teaching strategies to maintain the active involvement of the trainees. The techniques used during the training will follow the fundamental concepts of effective adult learning theory and require extensive active participation of the trainees. A basic and important requirement of the training will be to give every member of the staff the tools he or she needs to gain respondent cooperation at every level of participation and to acquire the skills needed to combat nonresponse and promote continued response.

As part of the QA/QC plan, a training roster will be developed for each Study Center that will include the type of competency assessment or certification required for each Study Center staff person. Almost all of the training modules will require a competency assessment at the end of training before the trainee can begin data collection. For example, staff members who collect anthropometry data will be tested against the "gold standard" expert in a series of competency sessions at the end of training. Additionally some staff (e.g., phlebotomists and ultrasonographers) must have up-to-date certifications before they can begin data collection. The team responsible for the training will determine the competency criteria. As training and certifications are completed, the training roster will be updated to indicate the training and certifications received. The roster will be maintained through the IMS.

The QA/QC plan will include periodic staff retraining. Refresher training may be necessary to introduce new Study procedures and forms and to sharpen data collector skills. This will be done throughout the Study as a standardized means of delivering new information. The Study may identify a Study Center whose study staff members, when audited, are not passing standards or whose data do not correlate with standard examiners, and may decide to conduct refresher training at that Study Center. As the Study progresses, some attrition among Study Center staff is expected. This will make it necessary to train new staff. There may be a need for special training during the course of the Study, for example, to teach techniques for improving response rates among special populations (e.g., minorities, very young mothers, or single mothers), or to elicit feedback from interviewers on the effectiveness of outreach materials and the need for new items to target specific groups. Remedial training may be necessary when data collectors do not meet acceptable performance standards as identified using QC measures.

15.4 QA/QC for Data Collection Activities

QA/QC procedures will be developed and applied to all Study data collection and management activities including interviewing, taking physical and medical measurements, collecting and handling environmental samples and biological specimens, and processing the collected data. QA/QC procedures regarding maintenance and calibration will be developed and applied to the measurement equipment used in the Study. There will also be QA/QC procedures developed and applied to the environmental and clinical laboratories and testing facilities utilized.

All Study data will be carefully and thoroughly reviewed and edited for consistency and range checks. Inconsistencies, anomalies, and outliers will be identified, examined, and verified when necessary. For example, participant demographic characteristics will be checked against reported health conditions and medical events for logical consistencies, and blood pressure measurements will be checked for end-digit preferences. All data collectors will be directly observed, indirectly monitored, and evaluated for quality issues such as protocol adherence and inter-rater reliability measures.

Study staff will observe Study Center data collection staff to evaluate procedures and protocols during participant identification activities, while completing the interviews, while collecting specimens and samples, and while taking physical measurements, during the field pilot tests and dress rehearsals. After this initial period, Study staff will conduct at least one in-person audit in the field per data collector per year to monitor interviewing techniques and all other data collection activities. Data collectors will be observed while conducting the home visits as well as the clinic visits. Study staff will develop a standardized electronic form for use by auditors in evaluating performance during these observations.

The Study will assign senior staff, trainers, or trained designees to conduct the field audits. The field auditors will record the results of each audit item on the form and will use the completed forms as the basis for providing rapid feedback. Individual and/or group feedback may be provided. Completed observation forms will be kept for the duration of the Study and will be used to assist in identifying topics for review during refresher trainings.

The procedures described above will be applied to all data collection activities, but there will be additional QA/QC procedures developed and applied to each of the specific types of data collection activities. Sections 15.5 through 15.9 summarize these additional QA/QC procedures.

15.5 QA/QC for Interviewing

Re-interviews, or "verifications," will also be used to monitor interviewer work. The verification will confirm that the interview was conducted and verify a few selected responses. Verification QC will be conducted by telephone by Coordinating Center staff. Cases to be verified will be selected through the IMS as work is completed. All of an interviewer's work will be eligible for verification regardless of the final disposition. Typically, 10-15 percent of each interviewer's cases will be selected for verification. Only a certain number of highly objective questions will be selected for verification, both to reduce respondent burden and to protect against discrepancies due to legitimate response changes. Interviewers will be told their work will be verified but will not know the number of cases or the procedure for selecting cases. If at any time verification indicates the possibility of falsification, the Coordinating Center will begin a 100 percent verification immediately of the interviewers' work. The Coordinating Center will report verification rates and results through monthly progress reports.

Falsification will be further substantiated through the use of digital time stamp reports and tracking GPS coordinates. A systematic review of digitally entered time stamps for work done by each interviewer will be an important indicator of potential problems in the field. These time stamps will generate several reports that will be routinely reviewed by Study staff. Any unusual or suspicious pattern in the digital entry trail must be explained and will trigger a higher validation rate for the interviewer.

Study Center interviewers will be required to edit all work before finalizing the data collection case. After completing each case, the computer will display any outstanding data collection activities and exams which the Study Center staff would review and finalize. If the data are collected at a home or birth visit or some other facility, it will be further reviewed at the Study Centers before uploading to the IMS. If necessary, the interviewers will receive immediate feedback to rectify any problems. After this edit, the completed work will be uploaded to the IMS. There will be built-in editing procedures in the IMS that will support a further review of the data. For example, all text entries in the questionnaires, as well as other critical data items, will be reviewed. Whenever possible, the Study Center coordinator or an assistant will re-edit 10 percent of each interviewer's work.

15.6 QA/QC for Collecting and Handling Samples and Specimens

All procedures for the collection of environmental samples and biological specimens will have data collection forms specific to each specimen or sample to be collected. These forms will be developed to allow the monitoring of data quality across the Study. All procedures and corresponding forms will be evaluated regularly for effectiveness, and, if a modification is required, changes will be implemented seamlessly and the modification will be documented. On a periodic basis, all parties affected by the procedure and data collection forms will be solicited for any needed modification or update.

Observing or auditing the work of sample collectors will be done to evaluate procedures and protocols as described in Section 15.4. In addition, Study staff will observe sample labeling at the collection site and processing, storage, packaging, and shipment of biospecimens and environmental samples to ensure these activities are conducted according to Study procedures.

In addition to conducting visits to observe field procedures, the Study will establish a schedule for regular reviews of biospecimen and environmental sample data, problem logs, equipment logs, maintenance records, and calibration results for all field work. Review of biospecimen and environmental sample data can be considered an indirect observation, a variation on the method of direct observation that may be suitable for some collection tasks, either as a substitute for or supplement to the

field audits. During an indirect observation, field staff performance will be monitored after the activity is complete, for example, by review of data from completed collection forms and comparison of the collection data to the laboratory results of analyzed specimens. The resulting data can be used as a measure of the quality of data collection or specimen collection.

All Study Centers will be required to keep logs of reported problems with specimen and sample labeling, processing, transfer, and shipment. These logs will be maintained on the IMS, as would similar logs from the biorepositories and analytic laboratories. The Study will track these logs on the IMS to identify, investigate, and resolve these types of problems with the Study Centers, laboratories, and repositories and to make recommendations for modifying procedures as necessary.

All biospecimen and environmental sample measurement equipment used in the field will be required to have regularly scheduled maintenance and logs of the maintenance, operating status, and all calibration results. The written procedures will describe, in detail, calibration procedures for all biomedical and environmental measurement equipment. If a particular instrument is required to be calibrated prior to each use, the Study will specify these calibration tests as well. Study procedures will include instructions on how to handle situations where equipment does not meet the specified calibration criteria. Study Center staff will be trained to calibrate and maintain all instruments and equipment in accordance with the approved procedures, including equipment that may need to exceed manufacturer recommendations because of extensive use.

The results of equipment maintenance and calibration activities will be automatically tracked in the IMS. If missing logs, failed calibrations, drifting, or other problems are found, the Coordinating Center will contact the affected party to discuss and correct the problem. If needed, a site visit will be made to observe the questionable equipment and procedures.

The Coordinating Center will work with the NCS Program Office to develop procedures designed to address the need for resampling and duplicate or repeat collection of samples. These procedures could apply to collection of most biospecimens or environmental samples (e.g., more blood, urine, or breast milk, or another dust, air, or soil sample). The Study will identify quality control samples to be used, including specifications as to their content, number of samples to be obtained, possible sources, and assurance of the quality of the samples and specimens.

15.7 QA/QC for Environmental/Clinical Labs, Repositories, and Testing Facilities

The Study will require all laboratories to submit the standard operating procedures, which will be used for the NCS. These documents will be logged and evaluated to ensure the standard operating procedures are written in accordance with current guidelines and other regulatory requirements, as well as Study procedures. Revisions will be requested as needed. All current and past standard operating procedures will be submitted and maintained electronically in the IMS, which will be easily accessible and searchable by the NCS Program Office.

The Coordinating Center will work with the NCS Program Office to define and implement procedures for monitoring the performance of the laboratories, testing facilities, and repositories. The monitoring will continue throughout their performance. The performance monitoring will include implementing external QC through use of split duplicate and other QC samples and review of those results. Reports will be developed to ensure production standards are met; to identify inconsistencies and inaccuracies in specimen type or labeling; to identify results that fall outside of expected parameters; to identify any trends in analysis over time; and to review internal standardization and proficiency sample analysis conducted as part of accreditation or certification programs. On-site observation will be done to

verify that procedures adhere to the NCS procedures and to verify equipment calibration procedures and internal QC. The Study will institute a methodology for regularly receiving data from the laboratories (monthly or semimonthly) to ensure quality and production standards are maintained. The Coordinating Center will perform this task by verifying the laboratory QC data and production levels are within acceptable parameters set forth by the NCS Program Office. The Coordinating Center will provide the results of the verification process to the NCS Program Office on a monthly basis.

The Coordinating Center will generate data collection forms for all audits and data collection mechanisms. Based on the data elements collected, the Coordinating Center will generate reports for the NCS Program Office. The Coordinating Center will request the input from the NCS Program Office into what data elements would be needed for reports of varying types. Based on these requests, the Coordinating Center will ensure all data are collected in a timely manner and any discrepancies will be reconciled.

The Coordinating Center will submit the audit procedures for each type of facility to the NCS Program Office to approve prior to any site audit. The Coordinating Center will arrange for and oversee audits of all laboratories and repositories before samples are sent, and every six months thereafter. All laboratory audit inspectors will have initial training and refresher training for current guidelines and will maintain training levels throughout the Study.

The audit staff will conduct six-month on-site laboratory reviews. Prior to the audit visit, the Coordinating Center will work with the NCS Program Office to address any particular concerns for the specific site to be audited by developing a site-specific audit plan. During the audit visit, the audit staff will operate from the approved standard operating procedures as well as from the site-specific audit plan of all Study-related equipment calibration documentation, internal assay QC specimen or sample results, and environmental control logs. The audit team will verify all components of the Study-related procedures conducted at the site, including staff training, procedures, security, environmental monitoring. The team will document any deviations or violations uncovered, as well as the corrective actions the site implemented to rectify them. The team will also work with the sites to obtain copies of all necessary documents and maintain these documents as a tracking mechanism for site performance. The Coordinating Center will document all findings and report to the NCS Program Office within one month of the site visit.

The Study will develop and implement procedures to work with laboratories to improve performance on an as-needed basis. The Coordinating Center will submit the procedures to the NCS Program Office for approval prior to implementation. The Coordinating Center will draft a procedure that addresses distributing QC specimens or samples. The procedure will address methods for distribution from the source to the Coordinating Center, the Study Centers, and the central repository, including sample handling and storage, as appropriate. For stable samples and analytes, many QC specimens may be ordered and sent out at one time; however, for unstable samples, there may need to be a steady stream of QC samples shipped out to the various entities.

The IMS will also specify the frequency with which each Study Center will insert the QC specimens and samples into the sample stream. This will primarily be by affixing a bar-coded label to each QC specimen or sample as if it were an actual Study specimen or sample during collection of actual specimens or samples. No sample type identifying information will be provided to the laboratories, (e.g., for environmental samples), and the surface area or volumes will not be provided. This will help prevent the laboratory from knowing which specimens or samples are QC checks.

The Coordinating Center will ensure that all QC specimens and samples are tracked in the IMS regardless of the source of the material. Ideally, the sources would enter the specimen/sample

information directly into the IMS. The IMS will have a specimen and sample tracking system (STS) component to track the shipment, handling, and results for all specimens and samples. The STS should have a set of specimen/sample ID numbers for QC specimens and samples that look like actual sample numbers.

15.8 QA/QC for Physical Measures Data Collection

QA/QC measures will include periodically reviewing equipment logs, maintenance schedules, and calibration results. Study staff will conduct any duplicate data collection specified in the Study documents, will ensure that all data collection forms are completed accurately in the field, and that all data such as ultrasound images and digital photographs are collected, labeled, and transmitted in accordance with specified study procedures. Study staff will document data maintenance efforts in the form of log files, summary operating procedures, and logs of changes to data.

Additional on-site observation audits of testing may include duplicate or repeat tests. Duplicate data will be collected on participants as part of the data collection protocol when the data are recognized as difficult to obtain. For example, in a typical ultrasound exam, each of the images will be taken twice to have at least two measurements of each type. Blood pressure measurements will be measured three to five times following a specific resting period. All measurements will be captured to allow an average reading to be computed based on an algorithm determined by the data analyst.

Gold standard examinations may also be used to measure the agreement between a recognized expert and an examiner by conducting examinations on the same participant during a single examination session. This type of QA is particularly relevant to some of the clinical examinations such as anthropometric measurements. The number of gold standard examinations required to assess the level of agreement, as well as acceptable levels of agreement, will be specified. The IMS will have the capability to generate a report that displays a side-by-side comparison of results from the primary and gold standard examinations. The gold standard examiner would be able to print and use this report to provide immediate feedback to the primary examiner. There should also be a program in the IMS that Study Centers run to produce inter-rater reliability statistics. If statistically significant differences between the gold standard and the primary examiner are identified, these will be addressed through retraining.

Replicate examinations, in which a second examination is performed on a participant by the same examiner as the primary examination, may be used to measure intra-examiner reliability on some clinical exams. Although replicate exams provide a good measure of reliability, they are burdensome for the participant and time consuming for the Study Center. Replicate examinations require that the participant return to the examination center (or the examiner return to the home) at a later date for a second-day examination. Not all exam procedures will be slated for inclusion in the second-day examination, and only those components that require this level of QA will be included.

15.9 QA/QC Activities for Data Management

The general approach to QA/QC for data management will be to rely on the approved procedures for ongoing structure to the program. Each procedure will include steps to facilitate identification of issues as they arise, support tracing problems to the source, and determine whether changes to procedures and/or the IMS will mitigate such problems in the future. QA/QC steps will be an integral part of each procedure and will be reviewed on an established basis but no less than annually.

The Coordinating Center will work closely with the NCS Program Office to ensure that NCS data are handled, processed, and managed with the highest level of quality. For data coding, the Coordinating Center will ensure metadata entry and maintenance, data review, and other manual data preparation procedures are performed properly. The Coordinating Center will ensure staff are trained and certified prior to beginning work and data management processes are designed specifically to identify errors resulting from data collection instrumentation or data processing activities.

A random sample of coders and data entry staff work will be reviewed by a second, more senior data management staff member or supervisor. Should consistent issues be identified, the staff member will be given additional guidance and training. If this guidance and retraining is not effective, the staff member will be removed from the project.

Automated edit software included in the IMS will be employed to detect item value, range, and inter-item logical inconsistencies, and checks will be implemented against historical data collected for the case. Results and resolutions of these edits will be maintained in automated form in the IMS. Any updates to NCS IMS databases as a result of data review will be accompanied by an annotation that includes the reason for the change, prior value, date of change, and authorization for the change, if required. Data management procedures will be fully documented and the documentation maintained online, accessible to data management staff. A log of all exceptional data management events will be maintained automatically within the IMS to support historical data questions.

Digital images will be used in several aspects of data collection such as radiology, pathology, or photography. Management of the digital images will include procedures for obtaining, transmitting, and storing images at the Study Centers and ultimately in image libraries in the IMS overseen by the Coordinating Center. A QA/QC protocol for management of digital images will be developed.

Approval guidelines for digital imaging equipment used in the Study will be developed. Study-approved protocols for technical parameters and measurements, calibration procedures and certifications, routine QC testing, and maintenance checks will all be addressed. Study Centers will be encouraged to participate in QA programs and certification programs for diagnostic imaging used in health care settings and will be expected to provide evidence of certification.

Procedures that ensure the capture of acceptable images for use in the NCS and the monitoring of images will be developed. Image transmission QC procedures that monitor the flow of complete image sets within the Study Centers and after transfer to the libraries will be addressed. Confidentiality and accuracy procedures for de-identification and anonymization of digital images, as well as ensuring accuracy in cataloging images, will be detailed in the QA/QC protocol. Evidence of robust backup, archival, and disaster recovery procedures will be required. Image files will be expected to be made available and accessible on secure Web sites.

Chapter 16

Sub-Studies, Outside Additions to the Core Protocol, and Adjunct Studies

16. SUB-STUDIES, OUTSIDE ADDITIONS TO THE CORE PROTOCOL, AND ADJUNCT STUDIES

As the National Children's Study proceeds, it will serve as a platform on which to build additional scientific research. Aspects of the NCS will yield ideas for sub-studies within the core protocol planning process and opportunities for adjunct studies, as well.

16.1 Sub-Studies Within the Core Protocol

The core protocol was developed through the NCS Program Office protocol planning process and paid for with NCS funds. This core protocol is to be performed on the entire cohort. Additionally, there will be some "sub-studies" of the core protocol planned by the NCS protocol planning team but performed on just a portion of the cohort. Sub-studies are generally funded by NCS and planned and approved through the same process as the core protocol. The Study Centers will carry out a sub-study as an integral portion of the core protocol.

16.2 Outside Initiated and Funded Studies That Pertain to the Entire Cohort

Outside initiated and funded proposals for the entire cohort will be considered as proposed modifications of the core protocol, ultimately decided upon and incorporated into the protocol by the same process that is used for the core protocol planning. The review process for such proposals will be a combination of the adjunct study review process and the core protocol planning process, as appropriate for the specific proposal.

16.3 Adjunct Studies

An adjunct study is performed on a portion of the cohort at one or more Study Centers, on all or a portion of their Center participants and/or their biospecimens or environmental samples. Adjunct studies are derived from or initiated and planned outside the NCS Program Office protocol planning process (e.g., can be initiated by a Study Center, government agency, independent investigator, industry, etc.), and are funded by such mechanisms as government grants applied for by the initiator, public-private partnerships, etc. Adjunct studies are reviewed and approved through a defined process and are implemented with the concurrence of the specific involved Study Centers. Individual Centers have the option of not participating in any particular ("outside initiated") adjunct study. Adjunct studies, therefore, are considered optional for the Study Centers.

While adjunct studies are generally neither planned by the NCS protocol planning team nor funded by NCS, in very specific circumstances the NCS may require or authorize and fund specific adjunct studies (for a portion of the cohort) to be planned "outside" the regular protocol planning process yet paid for with NCS funds (e.g. specific studies at specific Study Centers). These are referred to as "Internal Adjunct Studies," to reflect their internal (NCS) direction and funding despite "external" (i.e., other than NCS protocol planning team) scientific development.

16.4 Review Process

Adjunct studies and other additions to the protocol will undergo a formal review to assure maintenance of the quality and integrity of the Study and to address scientific merit; scientific "fit" with the NCS; burden to the participant and the Study; risk and other human subjects' issues; etc. A brief preliminary application has been developed as well as an in-depth full application to assure attention to the quality of the proposal and also to facilitate the submission, review, and approval of such proposals. Both are electronic and allow for relevant sections of government grant applications to be "cut and pasted" into the form.

Chapter 17

The Health Insurance Portability and Accountability Act

17. THE HEALTH INSURANCE PORTABILITY AND ACCOUNTABILITY ACT

Requirements for the release of identifiable health information by covered entities (e.g., certain health providers, health plans, and health clearinghouses) were set forth by the Health Insurance Portability and Accountability Act (HIPAA) of 1996, which became effective on April 14, 2003.

The Coordinating Center will work with the Study Centers to ensure that authorizations to release identifiable health information meet the HIPAA requirements. These authorizations must include a description of the information that will be used or disclosed; who may use or disclose the information; who may receive the information; the purpose of the use or disclosure; the expiration date (if there is no expiration date, it must be explicitly stated as such); notice that the authorization may be revoked; notice that the information may be disclosed to others not subject to the Privacy Rule (redisclosures may not be protected); notice that an individual may refuse to sign the authorization (if any treatment or payments are conditional upon the individual's signing the authorization, the individual must be informed of this); and the individual's signature and date.

These requirements for authorizations may be combined in a consent form or may be a separate document. A Privacy Board (or IRB serving as a Privacy Board) may authorize the release of identifiable health information if additional risks are not created for Study subjects.

Chapter 18

References

18. REFERENCES

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Chapter 19

Revisions, Errata and Addenda

19. REVISIONS, ERRATA AND ADDENDA

This chapter documents revisions, changes, and additions to The National Children's Study Research Plan and Appendices, Volumes 1 and 2.

19.1 Revisions From Original to Version 1.1

The following revisions and additions were made to Volume 1 effective on June 20, 2007:

- Revised title of Chapter 6
- Deleted bullet point four on page 6-10, subsection "Summary of preconception visits for women with a high probability of pregnancy," Chapter 6
- Revised Table of Contents to reflect title change to Chapter 6 and addition of Chapter 19
- Added "Version 1.1" to title and changed date to "June 20, 2007"